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The Effects of Spermine and Spermidine on the Interactions of Human Factors IXa, Xa, XIa, and IIa wi

Caitlyn Menicucci

The Effects of Spermine and Spermidine on the Interactions of Human Factors IXa, Xa, XIa, and IIa with Anti-Thrombin III

Caitlyn Menicucci

HONORS PROJECT

Submitted to the University Honors Program
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Dr. Arthur Brecher; Chemistry, Advisor

Dr. Daniel Pavuk; Biology, Advisor

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Acknowledgements

The completion of my honors project would not be possible without the numerous individuals who were kind enough to contribute their time, skills, and expertise. Dr. Arthur Brecher opened up his lab to me in May 2012 and has allowed me to learn in a research environment. His mentorship has allowed me to grow as a student and a scientist. He has taught me beyond science and given me life lessons as well as encouraged my academic growth. I am grateful for the opportunity to work with him. Dr. Daniel Pavuk was my biology professor and enabled me thrive in academia. Dr. Pavuk has always been available whenever I have questions, has aided in my background research, and has been a primary support in my encouraging me in research and in my future. I appreciate the time he spent speaking with me and his willingness to help me be successful. Finally, Dr. Nancy Boudreau dedicated her time helping me understand how to do statistical calculations to determine accuracy. She has been the go-to person with all calculations and has even been willing to check my final calculations to determine if more experiment should be performed. Lastly, my family has been the most crucial piece in my success at BGSU and as a student. Despite being over two-thousand miles away, I know that they fully support all endeavors that I am taking.

Summary of Experiments (With Definitions)

The blood cascading system is crucial in the maintenance of homeostasis. This is pertinent within the field of biochemistry since the blood cascading system is directly affected by the activities of certain enzymes and proteins found in plasma that can affect blood clotting (Rand, 707). Changes in such proteins and enzymes and their binding abilities can be observed using electrophoresis.

All of my experiments use Heparin, a common anticoagulant that is used in hospitals. Protamine Sulfate performs oppositely of Heparin, inhibiting interactions between Human Factors and Anti-Throbin III, preventing the formation of complexes. These experiments tested two polyamines, Spermine and Spermidine, to determine if they act more like Heparin and promote complex formation or like Protamine Sulfate and inhibit such interactions. Specifically, I tested Human Factors XIa, Xa, XIa, and IIa and observed their interactions with Anti-Thrombin III in comparison to Heparin and Protamine Sulfate.

Some basic definitions* to remember while observing the data include:

-

Heparin: A compound that is found in the liver and different tissues in the body that promotes blood coagulation

Protamine Sulfate: Reverses the anticoagulant effect of Heparin that comes from fish sperm.

Eletrophoresis: a method used in clinical and laboratory research to separate molecules based on size and polarity. The movement of the molecules happens through gels.

Polyamines: involved in many genetic processes such as cell migration and proliferation.

Spermine and Spermidine: Polyamines that that bind to the back of phosophate backbones on nucleic acids.

*All definitions are cited in the references section.

Materials and Methods

Acarylamide, A-8887, Lot # 29H0142, N'N'-methylene-bis-acrylamide, M-7279, Lot # 1122H0107, Trizma Base, T-6066, Lot #1109H401, Sodium Lauryl Sulfate, L-3771, Lot #59H03281, Coomassie Brilliant Blue, B-0149, Lot #: 108H5080, TEMED, Lot # 59H1138, Heparin, H-3393, Lot # 69H1151, all come from Sigma Chemical Company (St. Louis, MO). Reagent Grade Methonal, Lot # 4228246 came from E.M. Science. Isobutyl Alcohol, CAR # 220-4 came from Baxter Healthcare (Deerfield, IL). Enzyme Grade Glycerol, G-33, Lot # 701544 from Fisher Scientific (Pittsburg, PA). Mini-PROTEAN 3 system short glass plates, CAT # 1653308, 10 Lane Well Comb, CAT # 1653354, Spacer Glass Plates, CAT # 1653310, Human Factor Xa, CAT # HCXA-0060, Lot # BB0927-0.1 MG, 9.2mg/mL, Human Anti-Thrombin III, CAT #: HCATIII-0120, Lot#: BB0419-1 MG, 7.2 mg/mL, Human Factor XIa, Lot # BB0326, 3.6 mg/mL,, Human Factor IXa Lot # BB0412, 7.1mg/mL, , Human Factor IIa, Lot#: AA1014, 9.0mg/mL, all from Haematologic Technologies, Incorporated (Essex Junction, VT).

The molecular weights of enzymes used are as follows: Xa- 46000 MW, ATIII-58000 MW, XIa- 160000 MW, IIa- 36700 MW, IXa- 45000 MW. All human factors were prepared for testing in similar ways. The major differences were in the gel preparation. Human Factors Xa and IIa used 10% acrylamide gels while Human Factors XIa and IXa used 7.5% acrylamide gels. Tris/NaCl buffer was added to the Human Factors for dilution. The dilutions were as follows: 1.59µL IXa in 13.4µL of Tris, 4.3µL of XIa in 14.6µL of Tris, 1.95µL Xa in 27 µL Tris, 3.60µL IIa in 18.4µL of Tris. The glass plates were cleaned with methanol and filled with the resolving gel. Resolving gels were made as follows: 7.5% Acrylamide gels: 4mL dH_2O, 2.5mL of 1.5M Tris/HCl pH 8.8, 2.5mL acrylamide solution, and 100µL of 10% SDS, 50µL APS, and 10µL TEMED. The 10% resolving gel: 4.1mL dH_2O, 3.2mL acrylamide stock

solution, 2.5mL Tris pH8.8, 0.1mL 10% SDS, 50µL 10% APS, 5µL TEMED. These gels were pipeted into the plates and polymerized for between 30 minutes and 60 minutes. Stacking gels were added on top of the resolving gels and lane combs were inserted into the stacking gel. The stacking gels were made as follow: 7.5% acrylamide gel: 3mL dH_2O, 1.27mL 0.5M Tris/HCl pH 6.8, 0.65mL acrylamide stock solution, 100µL 10% SDS, 25µL 10% APS, 5µL TEMED. The 10% stacking gel: 5.9 dH_2O, 1.5mL acrylamide stock solution, 2.5mL Tris pH 8.8, 0.1mL 10% SDS, 50µL 10% APS, 10µL TEMED. The stacking gels polymerized for between 30 and 45 minutes. The comb was removed and the gels and glass plates were put in the mini tank and 10X running buffer was added to the inner and outer chambers. Then, 5µL of each protein sample were added to their respective lane contents. The electrophoresis machine was turned on and ran for approximately 60 minutes for each experiment. The lane contents all included 2µL of ATIII and of each respective Human factor. One lane had 3µL of Heparin while another had 3µL of Protamine Sulfate. All lane contents incubated at room temperature for 30 minutes. Following incubation, 5X running buffer was added to stop further reaction and dilute. Then, the contents were boiled at 100 degrees Celsius for 2 minutes. This further ensured that all reactions had ceased. These mixtures were loaded into the gels. Each lane content is detailed in the cover pages for each Human Factor that was tested.

How to make Reagents

Methods

Reagents Prepared for SDS-PAGE experiment:
(Adapted from Coggin, 2003)

1) Acrylamide Stock Solution
    a. 29.2g acrylamide
    b. 0.8gN'N'-methylene-bis-acrylamide
    c. Take up in 100mL volumetric flask
    d. Filer via Vacuum filtration with Whatman #1 filter paper
    e. Store in a brown bottle or in the dark
2) 1.5M Tris/HCL pH8.8:
    a. Add 36.33g Trizma base and fill to 150mL with dH_2O
    b. Add 0.1M HCl droppwise to pH 8.8
    c. Bring to a total volume of 200mL
    d. Store at four degrees Celcius
3) 0.5M Tris/HCL pH 6.8:
    a. Add 6.055g Trizma base to 50mL dH_2O
    b. Ass 0.1M HCl to pH 6.8 then dilute to 100mL
    c. Store at four degrees Celcius
4) 10% SDS Solution (w/v):
    a. Take 10g Sodium Layryl Sulfate up in 75mL dH_2O
    b. Dilute to 100mL in volumetric Flask
5) 10% APS (Ammonium Persulfate):
    a. Add 0.1g ammonium persulfate and fill to 1mL with dH_2O
    b. Store at -20 degrees Celcius in 200 µL aliquots
    c. Prepare monthly for fresh solution
6) 10X Running Buffer:
    a. Add 30.3g Trizma Base
    b. Add 144g Glycine
    c. Add 10g SDS
    d. Bring to 1000mL with dH_2O, mix well but do not adjust the pH
    e. Store at four degrees Celcius
    f. If a precipitate forms, bring to room temperature before diluting with dH2O before use.
7) dH_2O Saturated Isobutyl Alcohol
    a. Extract isobutyl alcohol from large seperatory funnel after washing three times with dH_2O
    b. This solution must be stored out of light
8) Gel Equilibrium Solution A (30% Methanol, 3% Glycerol):
    a. Add 150mL methanol to 15mL glycerol
    b. Fill to 500mL with dH_2O in a 500mL volumetric flask

9) Gel Equilibrium Solution B (3% glycerol)

a. Add 15mL glycerol
b. Fill to 500mL with dH2O in a 500mL volumetric flask

10) 5X Sample Buffer
    a. Add 21.5mL glycerol
    b. Add 15mL of 0.5M Tris/HCL stock solution pH 6.8
    c. Add 10mL 10% SDS solution
    d. Add 3.5mL of 1% Bromophenol Blue (prepared in MeOH~ 1g BPB in 100mL MeOH)
    e. Total volume= 50mL
    f. This solution is added to protein sample to a final concentration of 1X
11) Coomassie Stain
    a. Add 0.5g Coomassie Brillian Blue to 250mL of ACS reagent grade methanol and mix well
    b. Add 200mL dH_2O
    c. Add 50mL glacial acetic acid
    d. Filter via vacuum filtration using Whatman #1 filter paper
12) Destain:
    a. Add 800mL dH_20
    b. Add 100mL ACS reagent grade methanol
    c. Add 100mL glacial acetic acid in a 1000mL volumetric flask
13) Tris/NaCl Buffer pH 8.0:
    a. Add 0.224g Trizma base
    b. Add 0.325g reagent grade NaCl
    c. Fill to 100mL with dH_2O
    d. Mix well and do not adjust pH
    e. Store at four degrees Celcius

Individual substances used and in what quantities:
-Anti-Thrombin III (4.10µg in 2µL; per lane)
-Human Factor IIa, Xa, XIa , and XIa (Depends on the human factor; included in the individual reports of the project)
-Protamine Sulfate (1.0µg in 3µL; per lane)
-Heparin (1.0µg in 3µL; per lane)

The above information was provided from materials in Dr. Arthur Brecher's on campus lab.

Significance and Hypothesis

While Heparin is a very commonly used compound, it may be beneficial in the future to find an alternative seeing how humans are becoming resistant to more and more types of drugs and antibiotics. By finding alternatives, we can also determine and guess how other compounds and proteins might behave within the blood cascading system. If it can be accurately determined that one a compounds acts more like another, then perhaps it would reacts to treatments the same, bind to similar compounds, or even act competitively.

My hypothesis is that Spermine and Spermidine will act more like Protamine Sulfate and inhibit the formation of the human factor-ATIII complex. I think that there may be slight formation of complex due to natural polarity, but as stated, I believe this complex will be significantly less than that formed in the heparin lane.

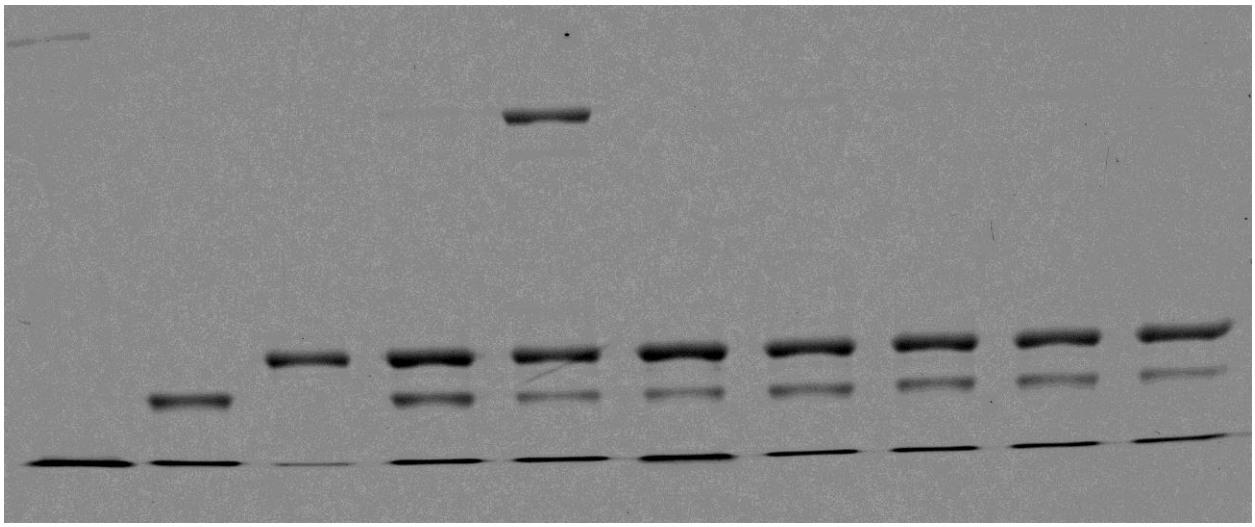
Experiments

The order my experiments are being presented in is the order in which I performed the experiments. All experiments include figures, which were calculated using IMAGEJ, a program designed to analyze gel electrophoresis bands by percent. You will also P-Values, which are calculations designed to determine the accuracy of the results presented.

First, will be Human Factor IXa with Anti-Thrombin III, followed by Human Factor Xa with Anti-Thrombin III, then Human Factor XIa wil Anti-Thrombin III, finishing with Human Factor IIa with Anti-Thrombin III.

IXa

Effects of Heparin, Protamine Sulfate, Spermine, Spermidine on the Interactions Between FIXa and ATIII [30’ Incubation]



1. 5µL Molecular Weight Markers+ 13µL Tris/NaCl pH8 + 5µL 5X Sample Buffer
2. 2.0µL (1.62µg) FIXa + 13µL Tris/NaCl pH8 + 5µL 5X Sample Buffer
3. 2.0µL (4.10µg) ATIII + 13µL Tris/NaCl pH8 + 5µL 5X Sample Buffer
4. 2.0µL (1.62µg) FIXa + 2.0µL (4.10µg) ATIII + 11µL Tris/NaCl pH8 + 5µL 5X Sample Buffer
5. [(2.0µL (4.10µg) ATIII + 1µg (3µL) Heparin) 15’ at RT, + 2.0µL FIXa + 8µL Tris/NaCl pH8] 30’ at RT, + 5µL 5X Sample Buffer]
6. [(2.0µL (4.10µg) ATIII + 1µg (3µL) Protamine Sulfate) 15’ at RT, + 2.0µL (1.62µg) FIXa + 8µL Tris/NaCl pH8] 30’ at RT, + 5µL 5X Sample Buffer]
7. [(2.0µL (4.10µg) ATIII + 0.07µg (3µL) Spermine) 15’ at RT, + 2.0µL (1.62µg) FIXa + 8µL Tris/NaCl pH8] 30’ at RT, + 5µL 5X Sample Buffer]
8. [(2.0µL (4.10µg) ATIII + 0.7µg (3µL) Spermine) 15’ at RT, + 2.0µL (1.62µg) FXIa + 8µL Tris/NaCl pH8] 30’ at RT, + 5µL 5X Sample Buffer] (0.1 Concentration of Spemine)
9. [(2.0µL (4.10µg) ATIII + 0.05µg (3µL) Spermidine) 15’ at RT, + 2.0µL (1.62µg) FIXa + 8µL Tris/NaCl pH8] 30’ at RT, + 5µL 5X Sample Buffer]
10. [(2.0µL (4.10µg) ATIII + 0.5µg (3µL) Spermidine) 15’ at RT, + 2.0µL (1.62µg) FIXa + 8µL Tris/NaCl pH8] 30’ at RT, + 5µL 5X Sample Buffer] (0.1 Concentration of Spermidine)

Photographs of the gels:
Figure A
Gel 1: (MWM on the left)

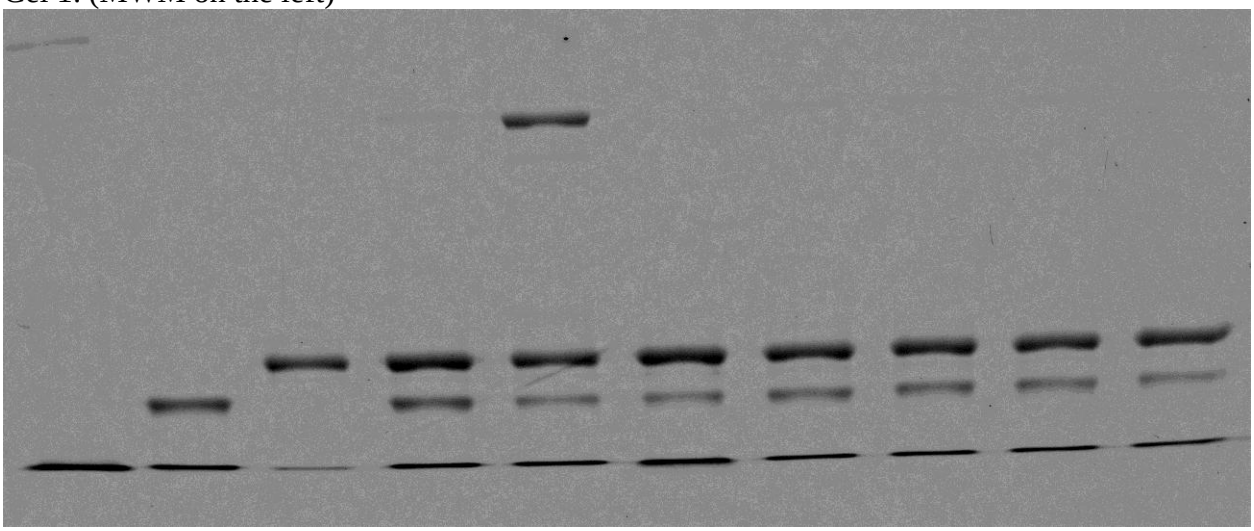


Figure B
Gel 2: (MWM on the right)

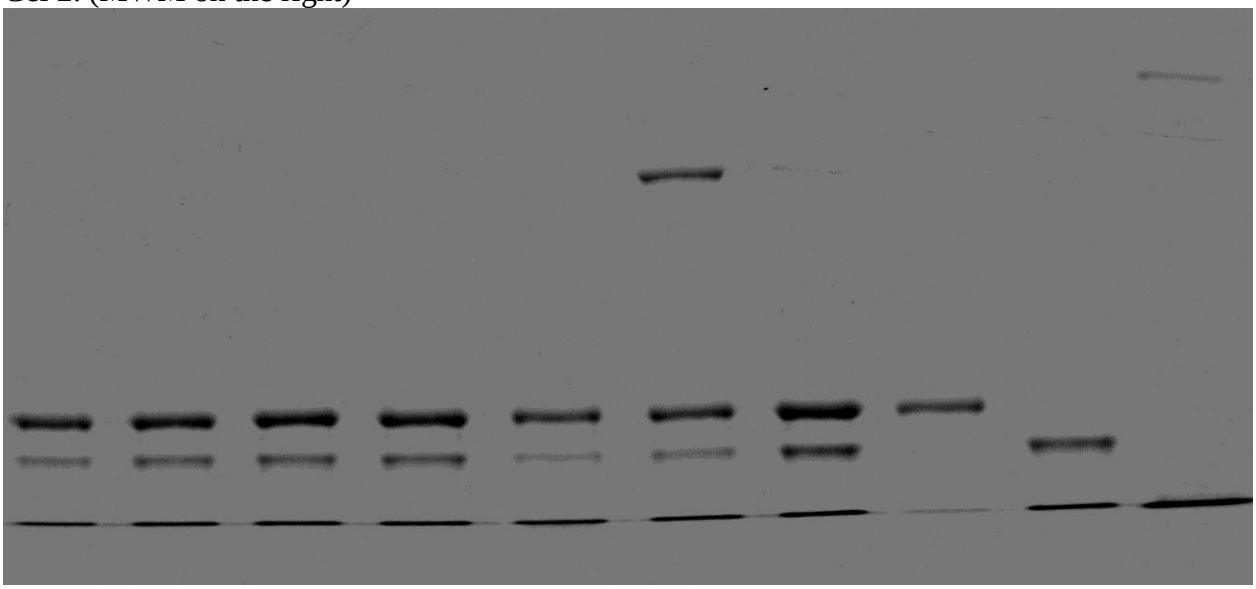


Table 1: Overall Protein Analysis Per Lane
*ND= Not detected

	1.62µg IXa and 4.10µg ATIII	1.00µg H	1.00µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
IXa-ATIII	2.235	29.57	*ND	1.125	1.47	1.16	0.476
Degradation	*ND	0.816	*ND	*ND	*ND	*ND	*ND
ATIII-M	45.89	35.18	49.74	49.08	50.12	50.98	55.44
ATIII	0.57	0.909	0.836	0.27	0.62	0.453	0.463
IXa	31.76	16.63	20.25	29.19	27.75	28.07	22.19
Solvent Front-	19.54	16.9	29.18	20.34	20.05	19.34	21.43

Figure C: Graphical Representation of Table 1.

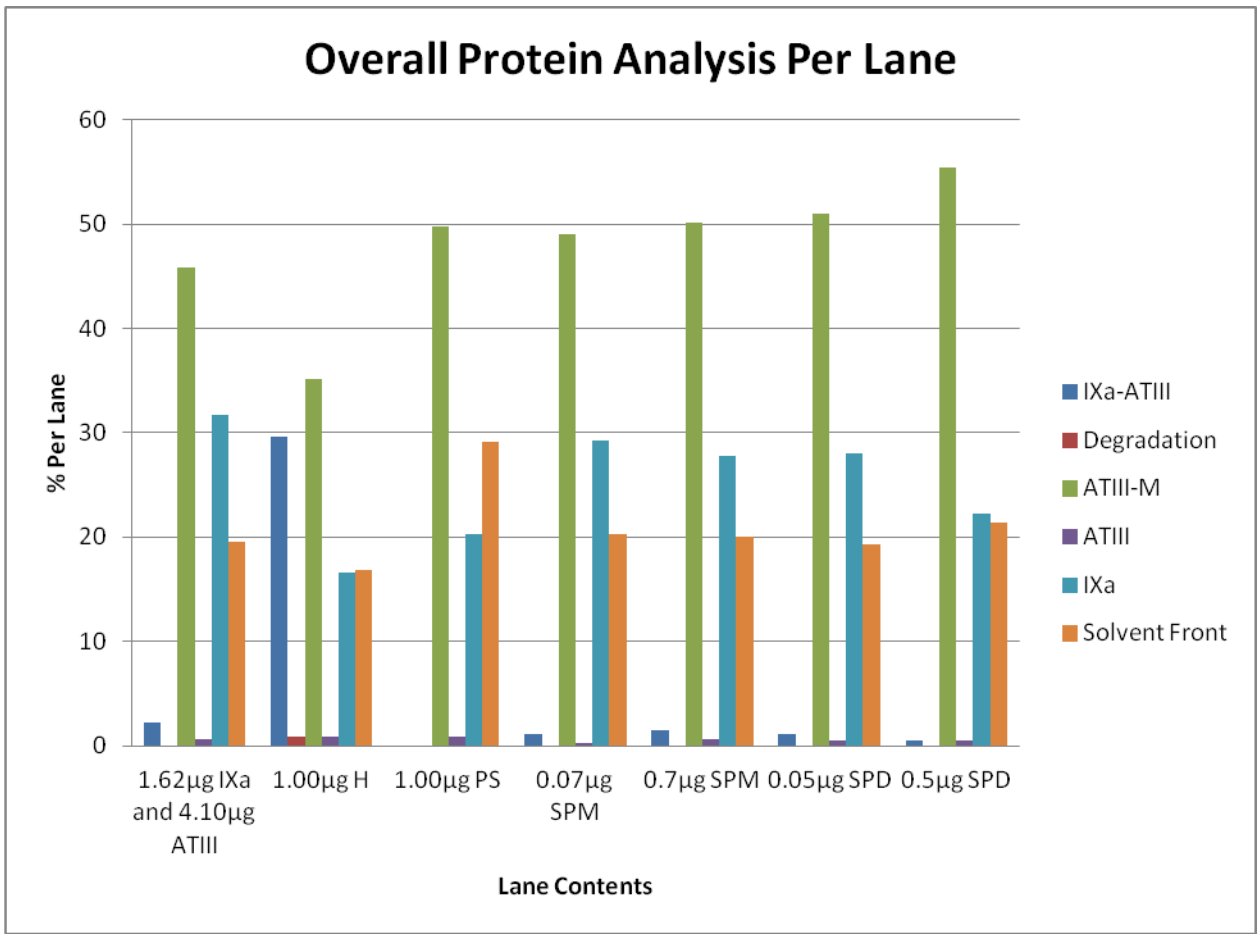


Table 2: IXa-ATIII Analysis Per Lane

	1.62µg IXa and 4.10µg ATIII	1.00µg H	1.00µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
IXa-ATIII	2.235	29.57	*ND	1.125	1.47	1.16	0.476

*ND=Not Detected

Figure D: Graphical Representation of Table 2

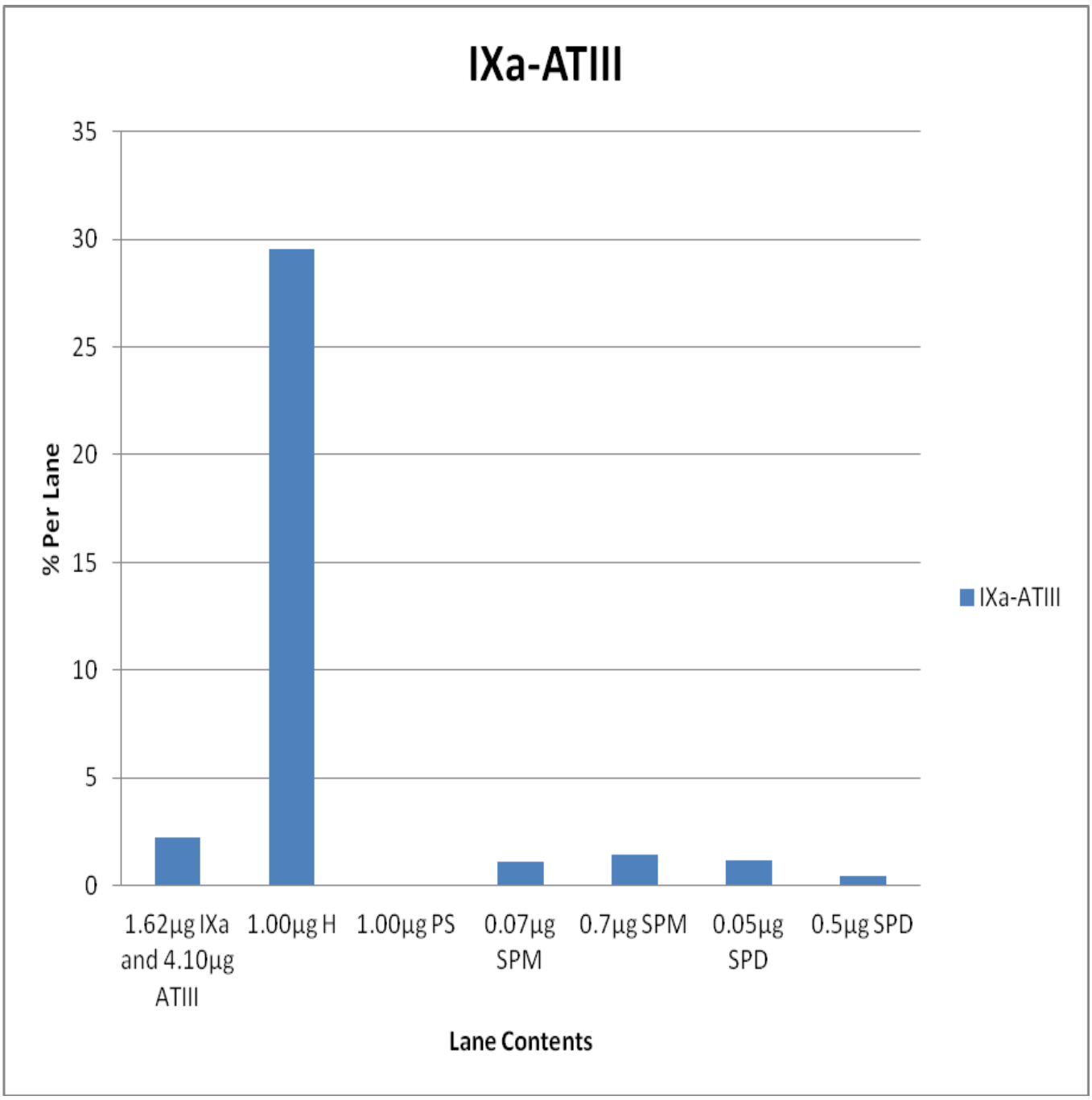


Table 3: Degradation Analysis Per Lane

	1.62µg IXa and 4.10µg ATIII	1.00µg H	1.00µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Degradation	*ND	0.816	*ND	*ND	*ND	*ND	*ND

*ND= Not Detected

Figure E: Graphical Representation of Table 3

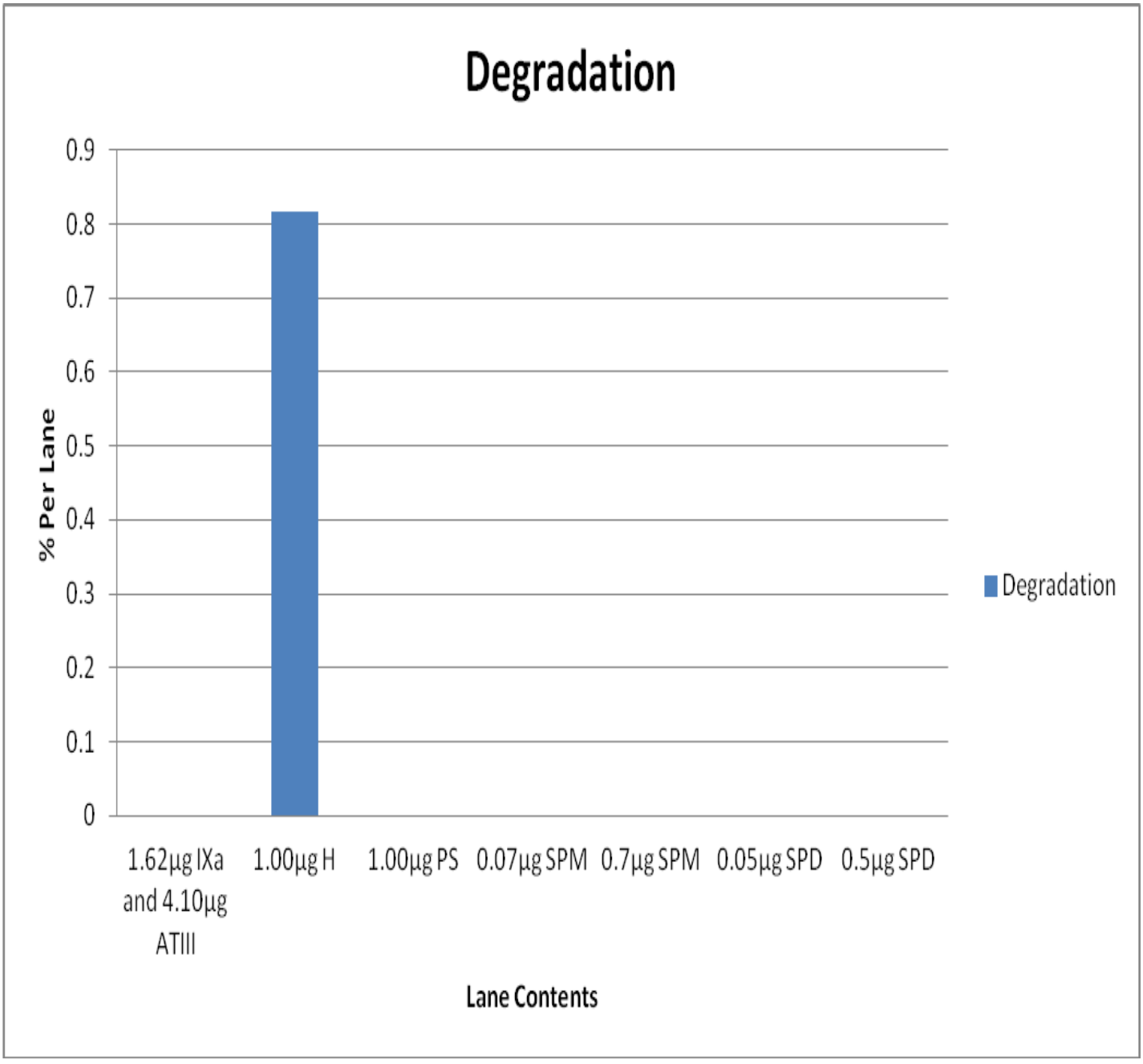


Table 4: ATIII-M Analysis Per Lane

	1.62µg IXa and 4.10µg ATIII	1.00µg H	1.00µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIII-M	45.89	35.18	49.74	49.08	50.12	50.98	55.44

Figure F: Graphical Representation of Table 4

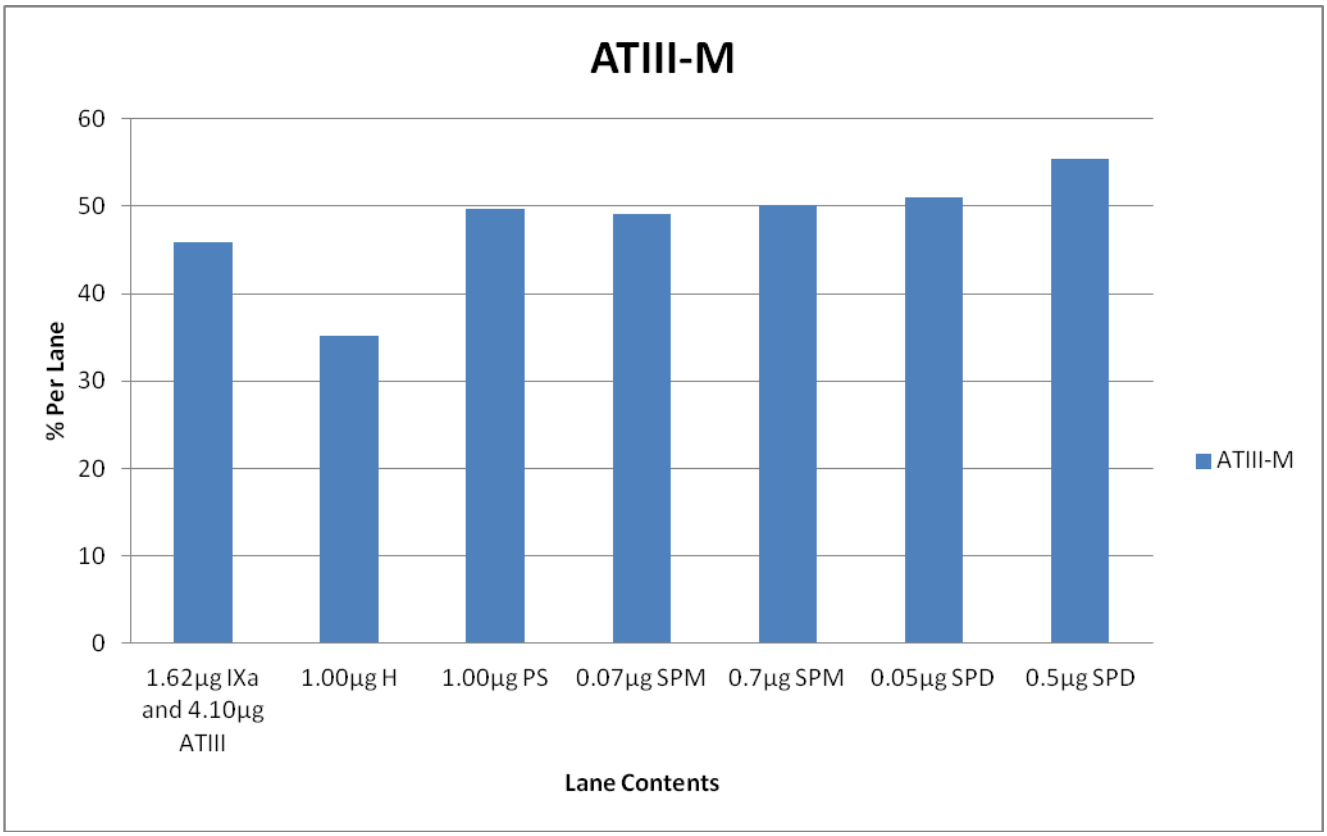


Table 5: ATIII Analysis Per Lane

	1.62µg IXa and 4.10µg ATIII	1.00µg H	1.00µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIII	0.57	0.909	0.836	0.27	0.62	0.453	0.463

Figure G: Graphical Representation of Table 5

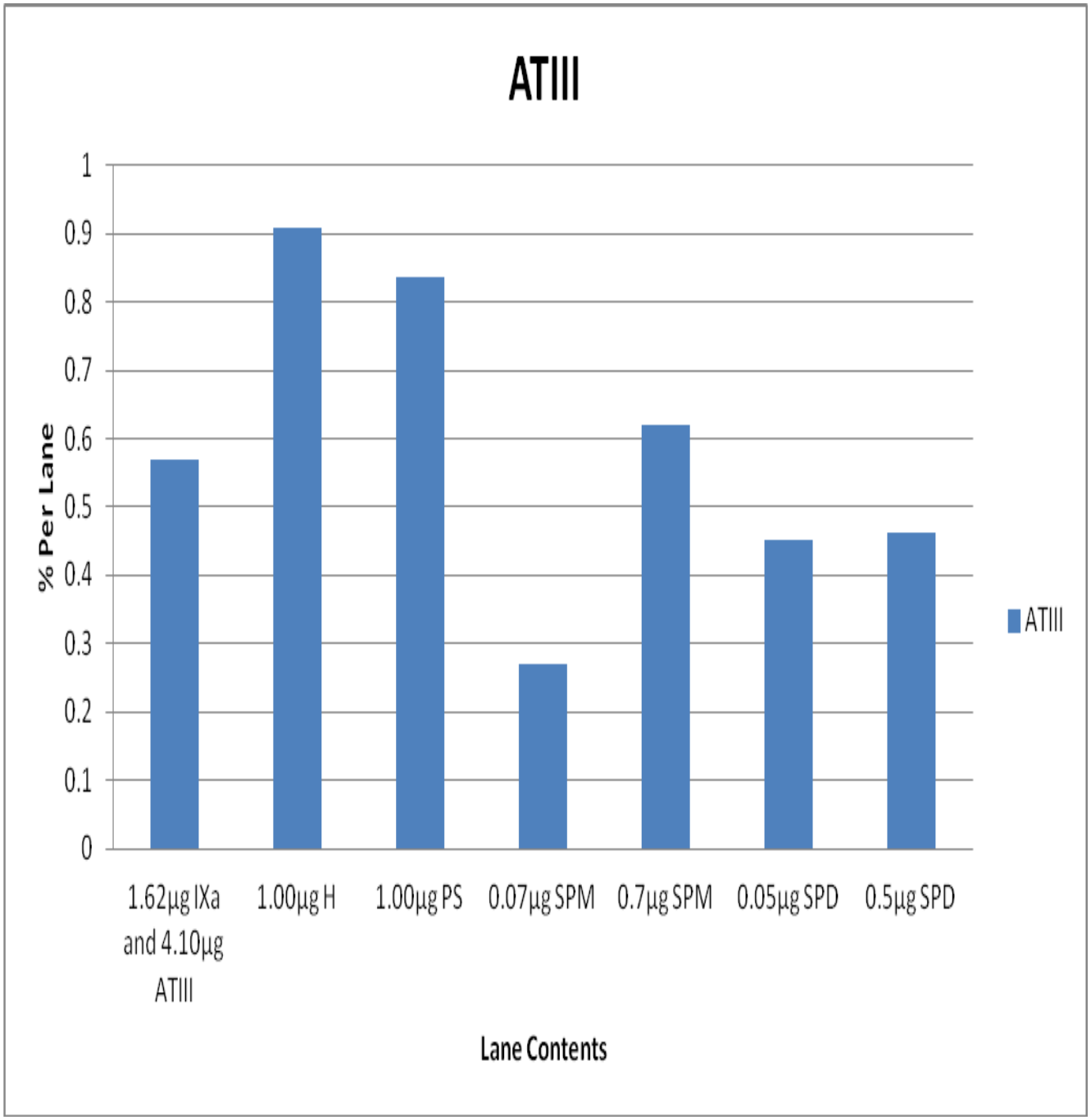


Table 6: IXa Analysis Per Lane

	1.62µg IXa and 4.10µg ATIII	1.00µg H	1.00µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
IXa	31.76	16.63	20.25	29.19	27.75	28.07	22.19

Figure H: Graphical Representation of Table 6

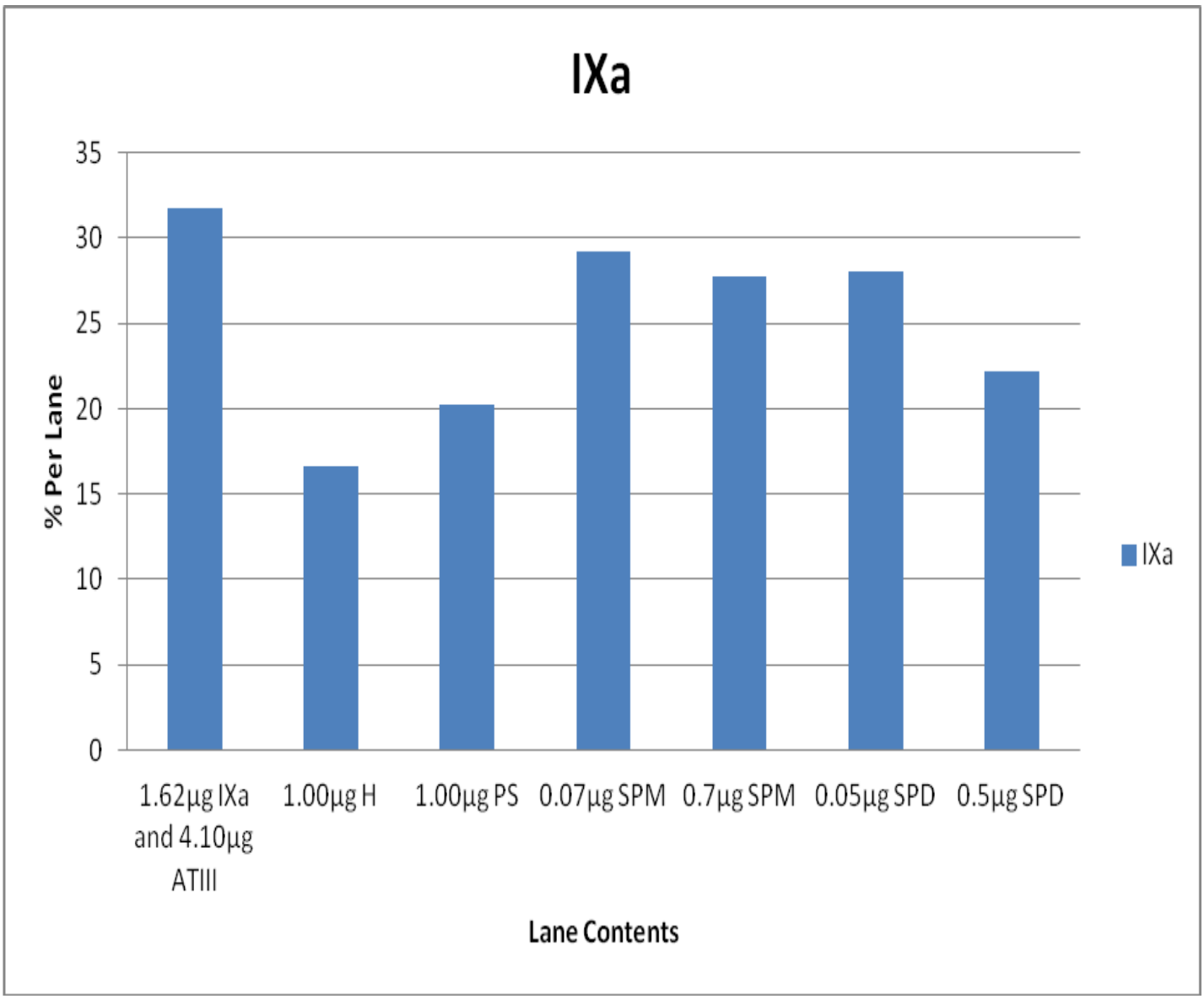
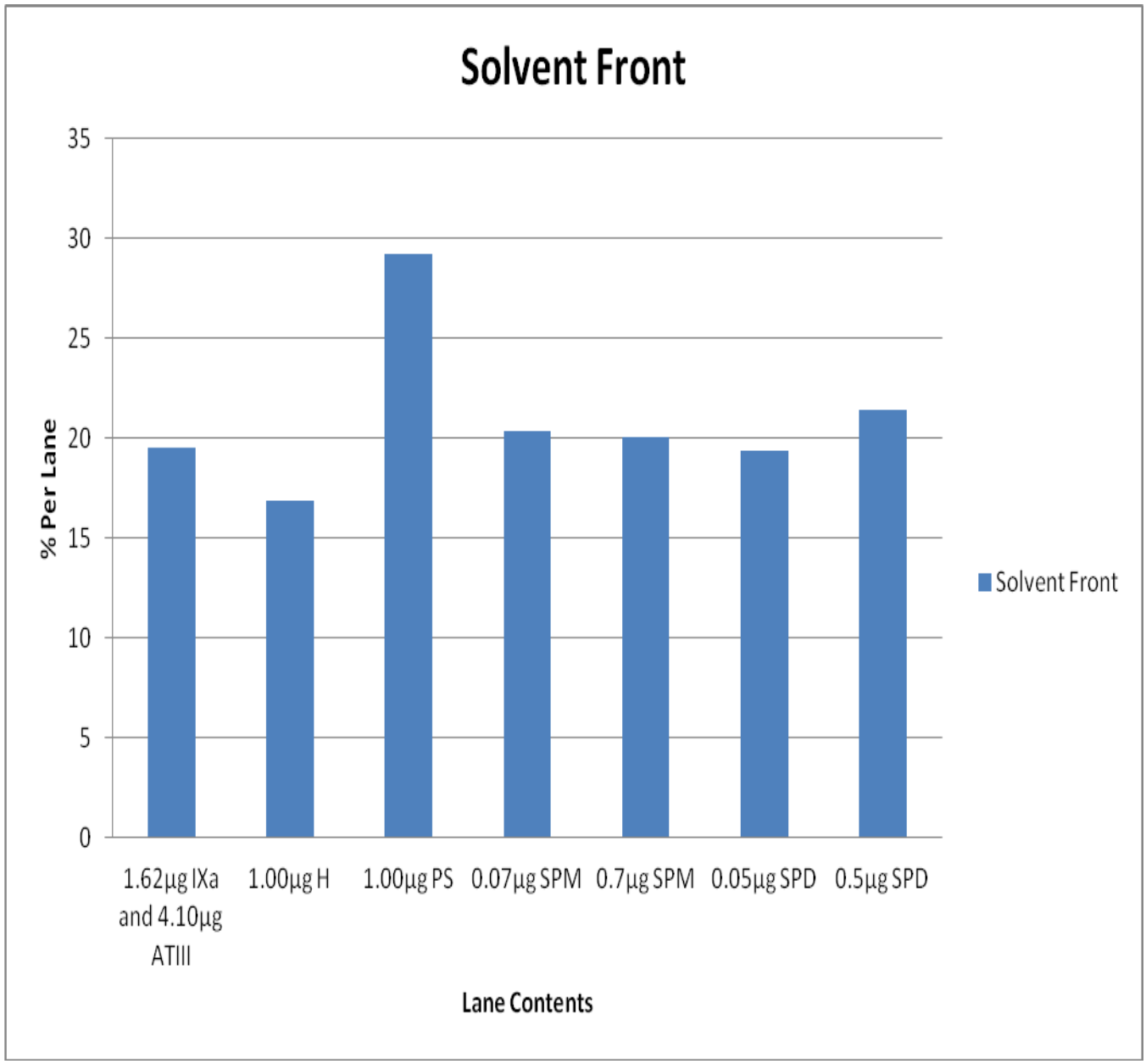


Table 7: Solvent Front Analysis Per Lane

	1.62µg IXa and 4.10µg ATIII	1.00µg H	1.00µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Solvent Front	19.54	16.9	29.18	20.34	20.05	19.34	21.43

Figure I: Graphical Representation of Table



Gel 2

Table 8: Overall Protein Analysis Per Lane

	1.62µg IXa and 4.10µg ATIII	1.00µg H	1.00µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
IXa-ATIII	2.299	29.28	*ND	1.05	0.95	0.69	0.973
Degradation	*ND	1.091	*ND	*ND	*ND	*ND	*ND
ATIII-M	46.93	38.21	56.65	49.77	50.08	50.4	56.11
ATIII	0.738	0.627	0.867	0.981	0.78	0.482	0.556
IXa	32.95	14.9	13.16	30.13	29.63	28.57	20.77
Solvent Front	17.09	15.89	29.33	18.08	18.57	19.86	21.59

*ND= Not Detected

Figure J: Graphical Representation of Table 8

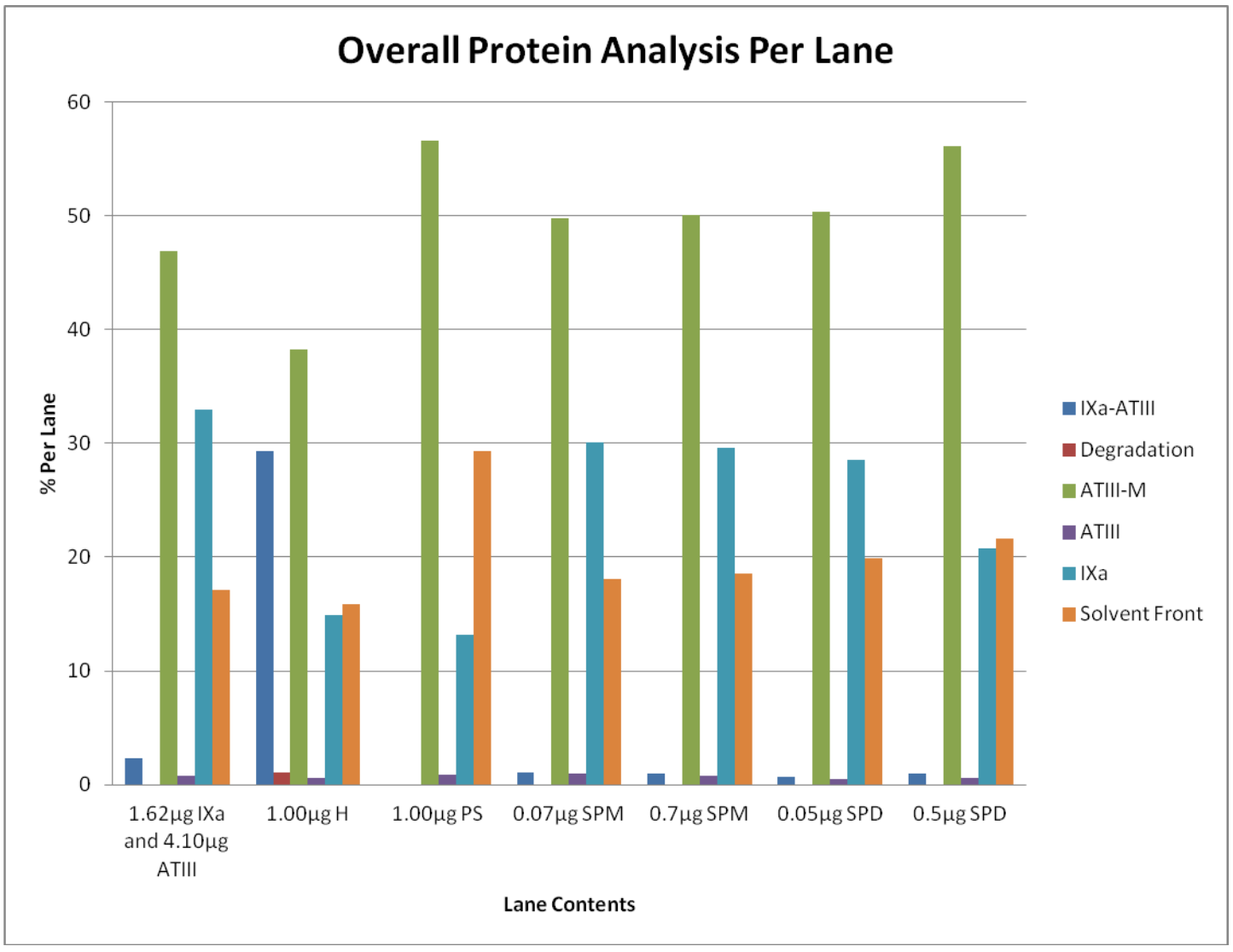


Table 9: IXa-ATIII Analysis Per Lane

	1.62µg IXa and 4.10µg ATIII	1.00µg H	1.00µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
IXa-ATIII	2.299	29.28	*ND	1.05	0.95	0.69	0.973

*ND= Not Detected

Figure K: Graphical Representation of Table 9

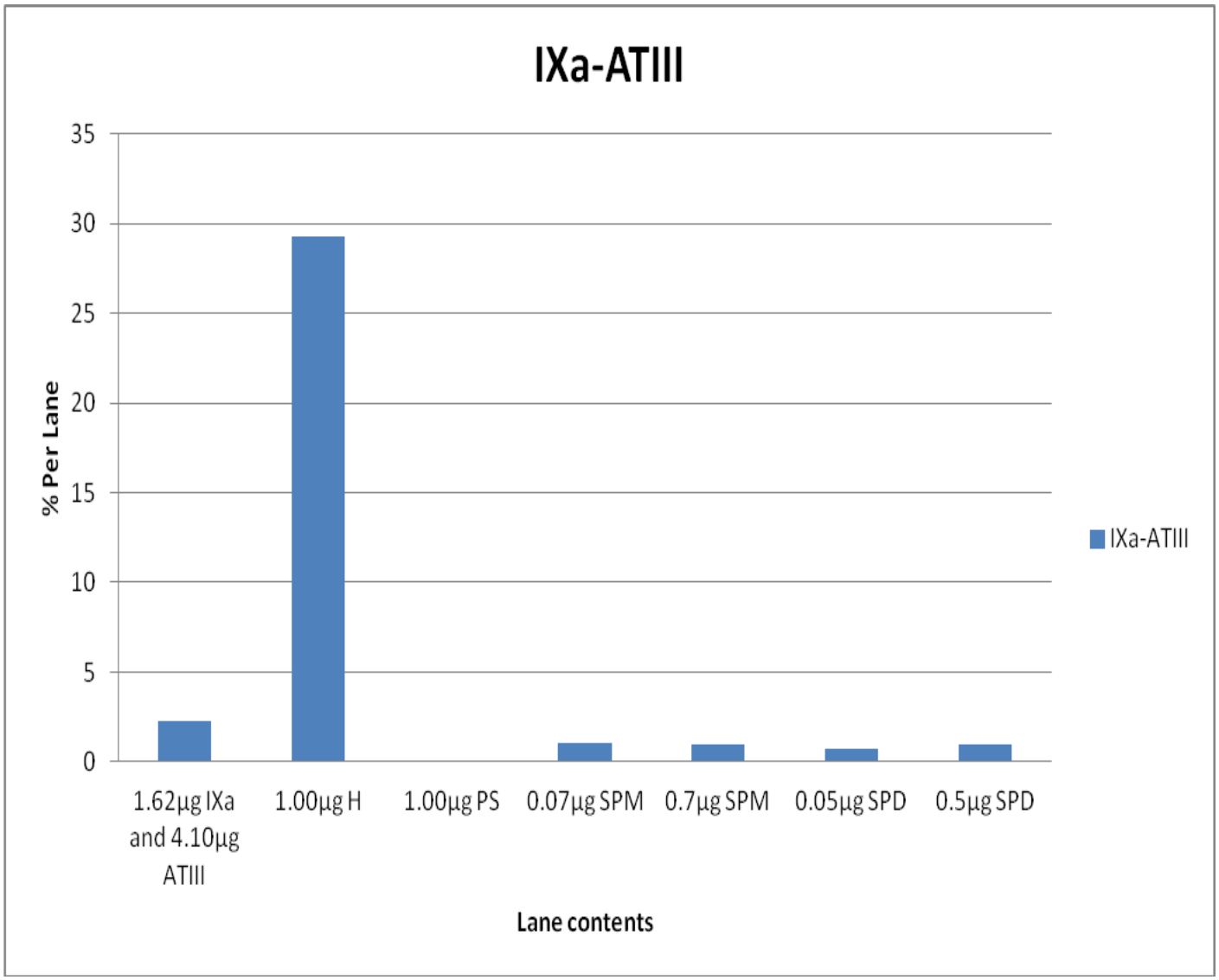


Table 10: Degradation Analysis Per Lane

	1.62µg IXa and 4.10µg ATIII	1.00µg H	1.00µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Degradation	*ND	1.091	*ND	*ND	*ND	*ND	*ND

*ND= Not Detected

Figure L: Graphical Representation of Table 10

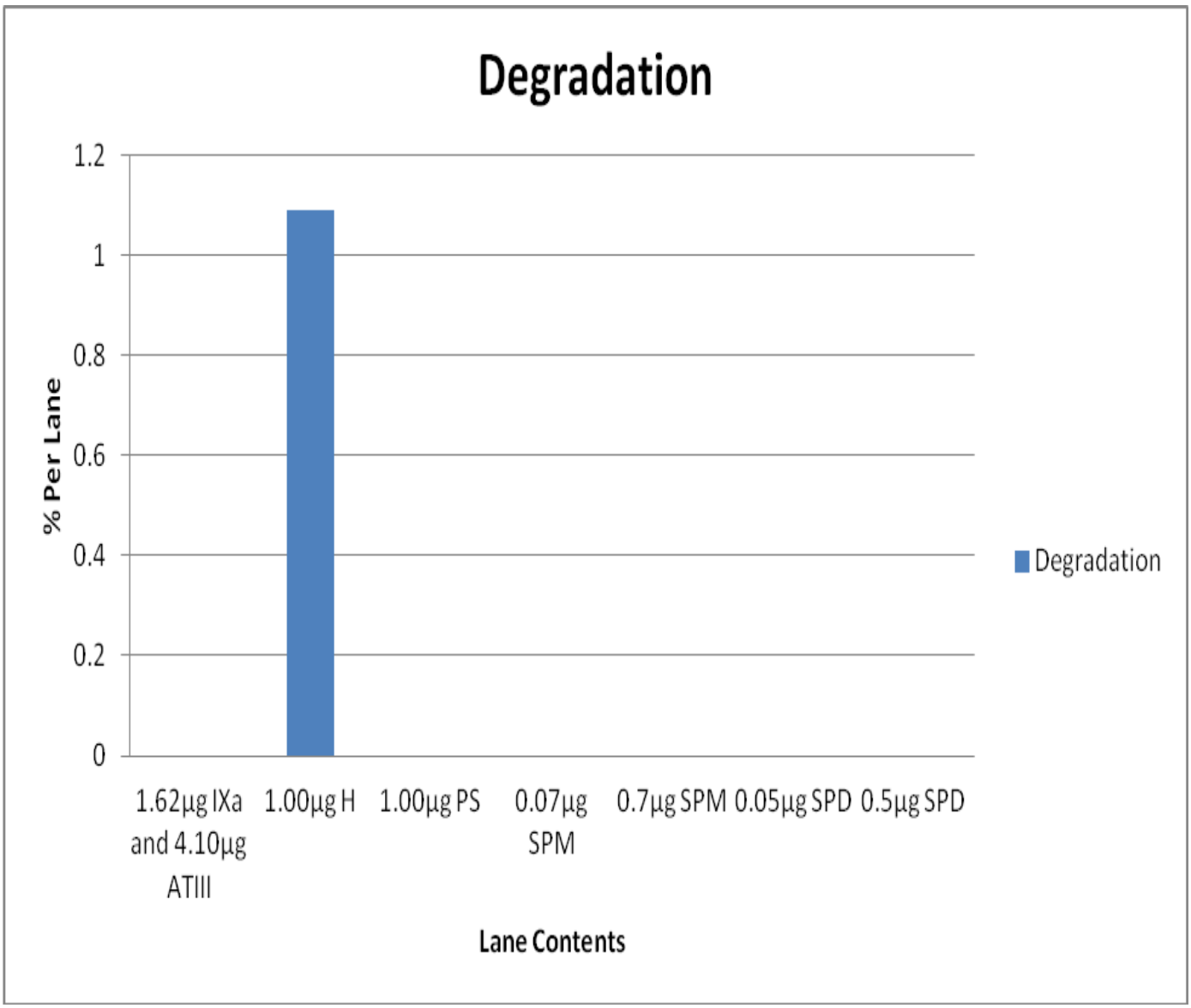


Table 11: ATIII-M Analysis Per Lane

	1.62µg IXa and 4.10µg ATIII	1.00µg H	1.00µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIII-M	46.93	38.21	56.65	49.77	50.08	50.4	56.11

Figure M: Graphical Representation of Table 11

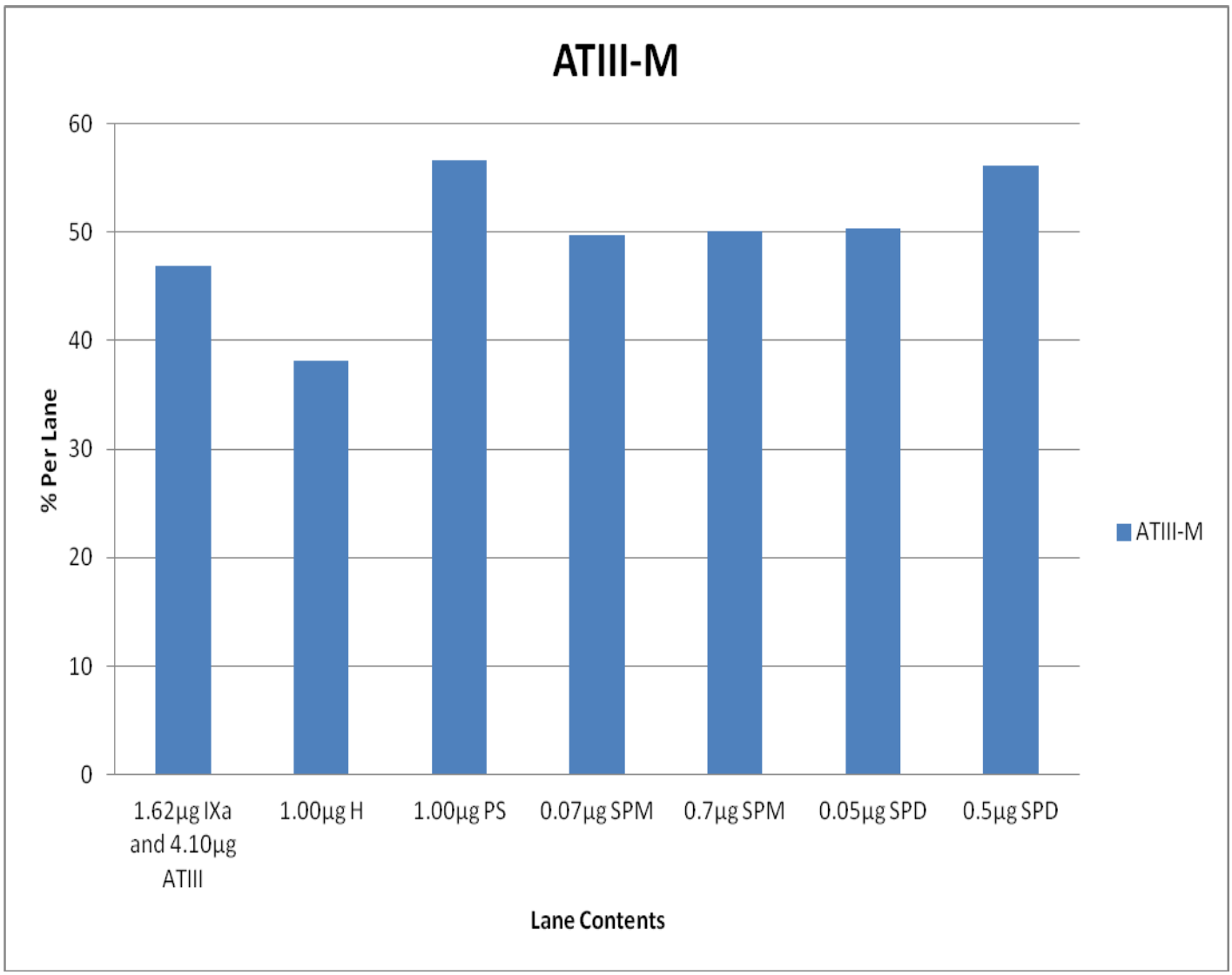


Table 12: ATIII Analysis Per Lane

	1.62µg IXa and 4.10µg ATIII	1.00µg H	1.00µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIII	0.738	0.627	0.867	0.981	0.78	0.482	0.556

Figure N: Graphical Representation of Table 12

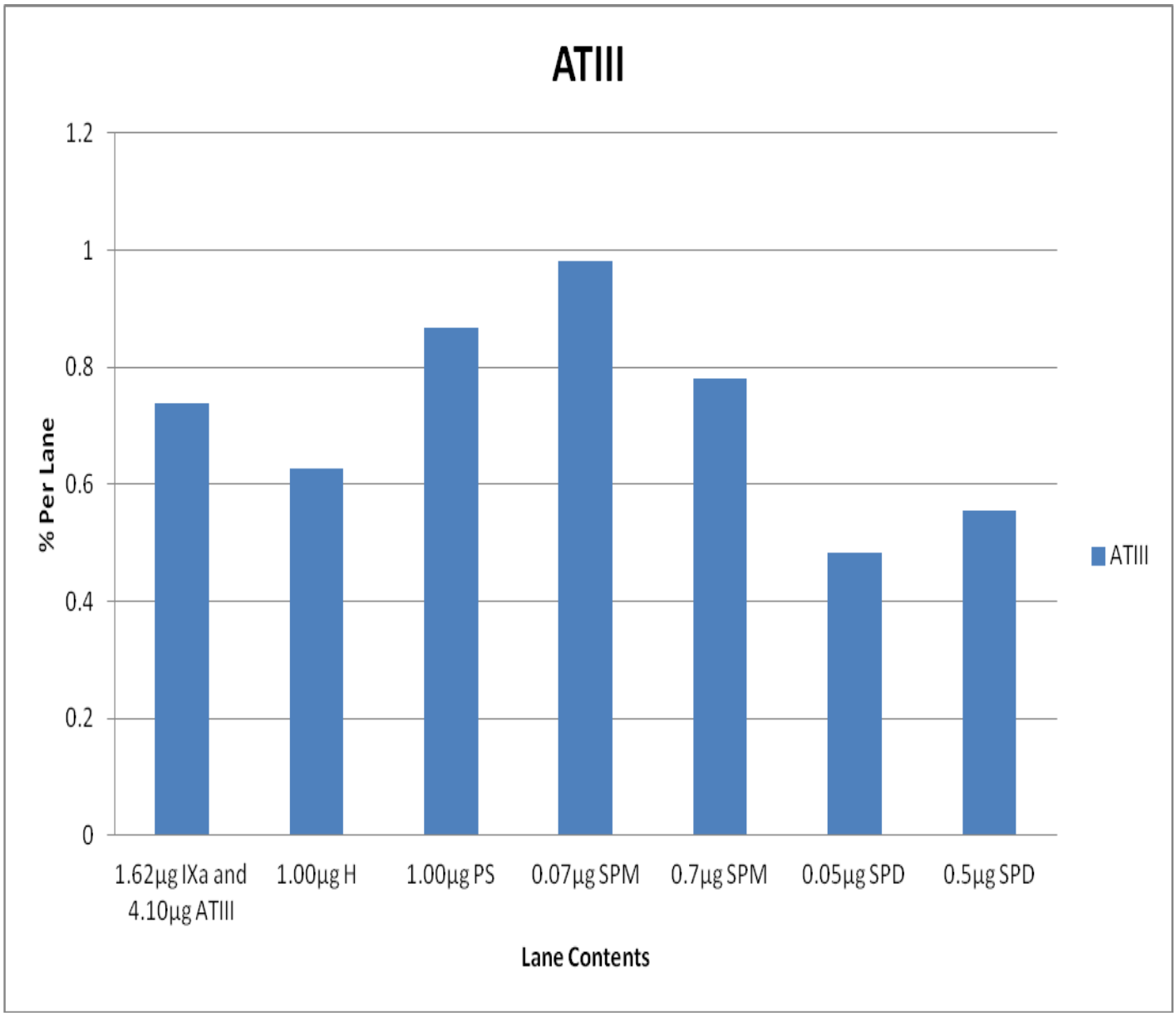


Table 13: IXa Analysis Per Lane

	1.62µg IXa and 4.10µg ATIII	1.00µg H	1.00µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
IXa	32.95	14.9	13.16	30.13	29.63	28.57	20.77

Figure O: Graphical Representation of Table 13

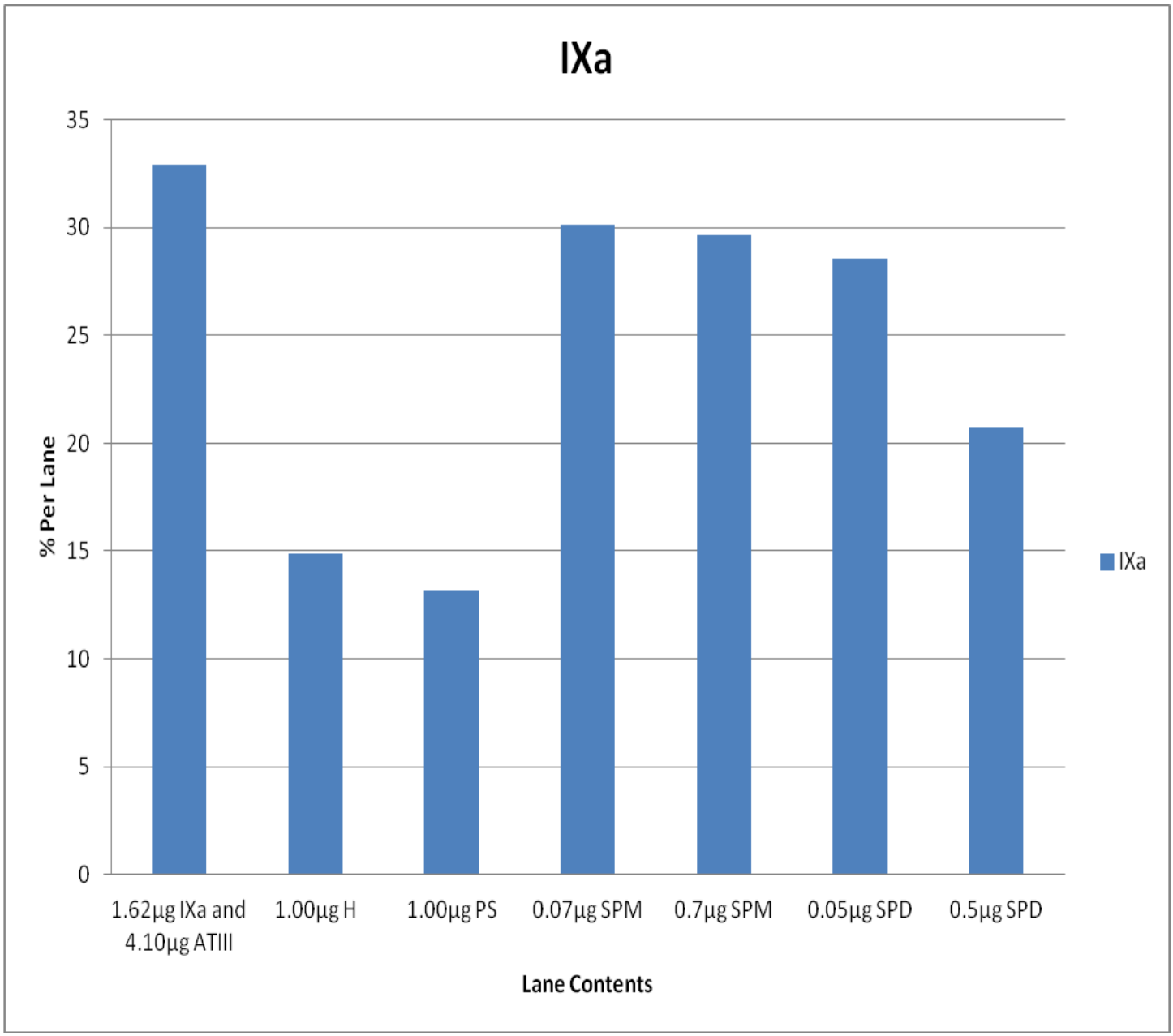


Table 14: Solvent Front Analysis Per Lane

	1.62µg IXa and 4.10µg ATIII	1.00µg H	1.00µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Solvent Front	17.09	15.89	29.33	18.08	18.57	19.86	21.59

Figure P: Graphical Representation of Table 14

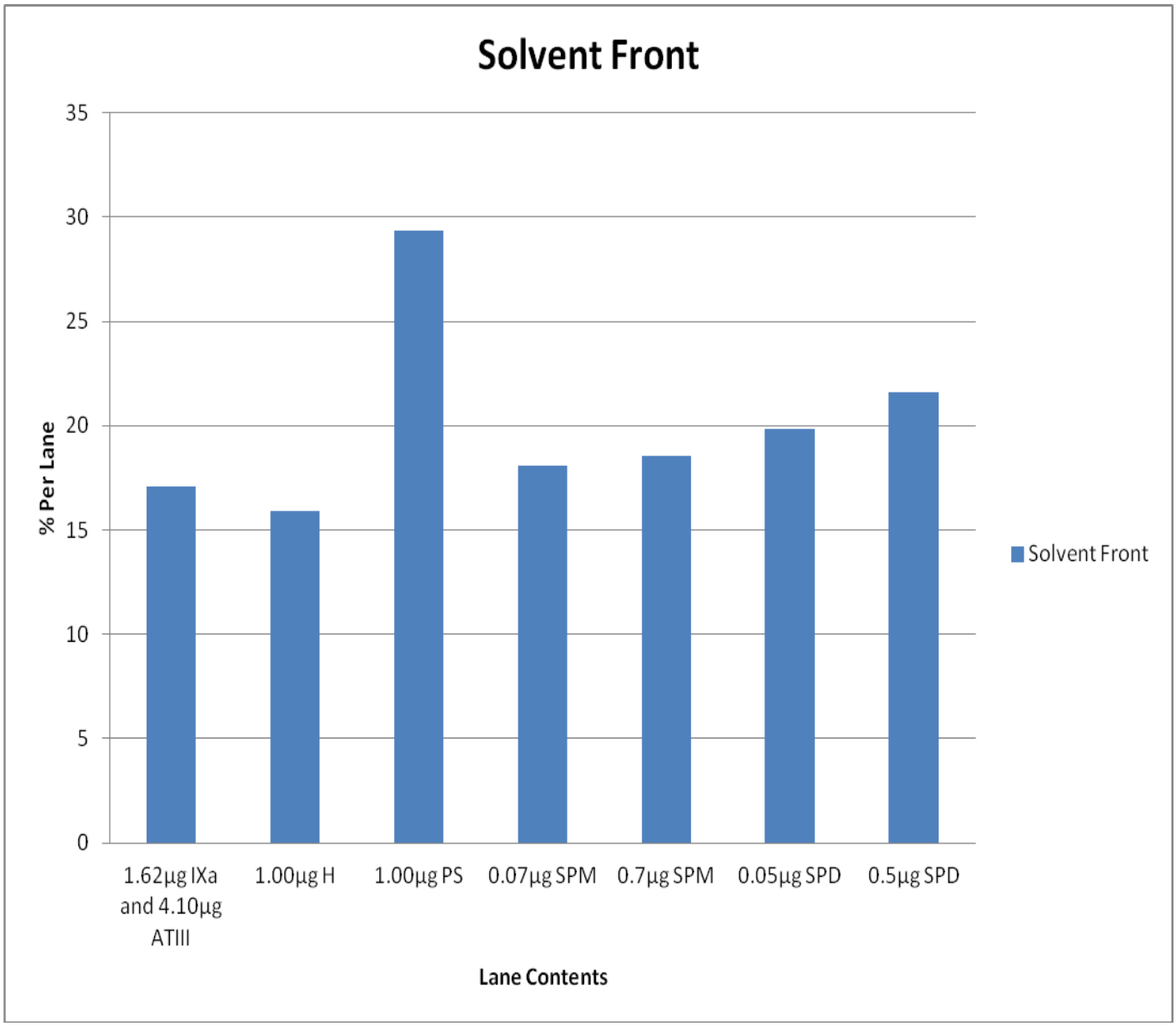


Table used for P Value Analysis:

	1.62µg IXa and 4.10µg ATIII	1.00µg H	1.00µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
IXa-ATIII	2.232	29.57	0	1.125	1.47	1.16	0.476
IXa-ATIII	2.299	29.28	0	1.05	0.95	0.69	0.973

P Values:

IXa-ATIII

	1.62µg IXa and 4.10µg ATIII	*1.00µg H*
Mean	2.2655	29.425
Variance	0.0022445	0.04205
t Stat	-152.1540616	
P(T<=t) one-tail	0.002091993	
t Critical one-tail	6.313751514	
P(T<=t) two-tail	0.004183987	
t Critical two-tail	12.70620473	

	1.62µg IXa and 4.10µg ATIII	*1.00µg PS*
Mean	2.2655	0
Variance	0.0022445	0
t Stat	67.62686567	
P(T<=t) one-tail	0.004706513	
t Critical one-tail	6.313751514	
P(T<=t) two-tail	0.009413025	
t Critical two-tail	12.70620473	

	1.62µg IXa and 4.10µg ATIII	*0.07µg SPM*
Mean	2.2655	1.0875
Variance	0.0022445	0.0028125
t Stat	16.5915493	
P(T<=t) one-tail	0.019161881	
t Critical one-tail	6.313751514	
P(T<=t) two-tail	0.038323761	
t Critical two-tail	12.70620473	

	1.62µg IXa and 4.10µg ATIII	*0.7µg SPM*
Mean	2.2655	1.21
Variance	0.0022445	0.1352
t Stat	3.596252129	
P(T<=t) one-tail	0.086330601	
t Critical one-tail	6.313751514	
P(T<=t) two-tail	0.172661202	
t Critical two-tail	12.70620473	

	1.62µg IXa and 4.10µg ATIII	*0.05µg SPD*
Mean	2.2655	0.925
Variance	0.0022445	0.11045
t Stat	4.99255121	
P(T<=t) one-tail	0.062924282	
t Critical one-tail	6.313751514	
P(T<=t) two-tail	0.125848564	
t Critical two-tail	12.70620473	

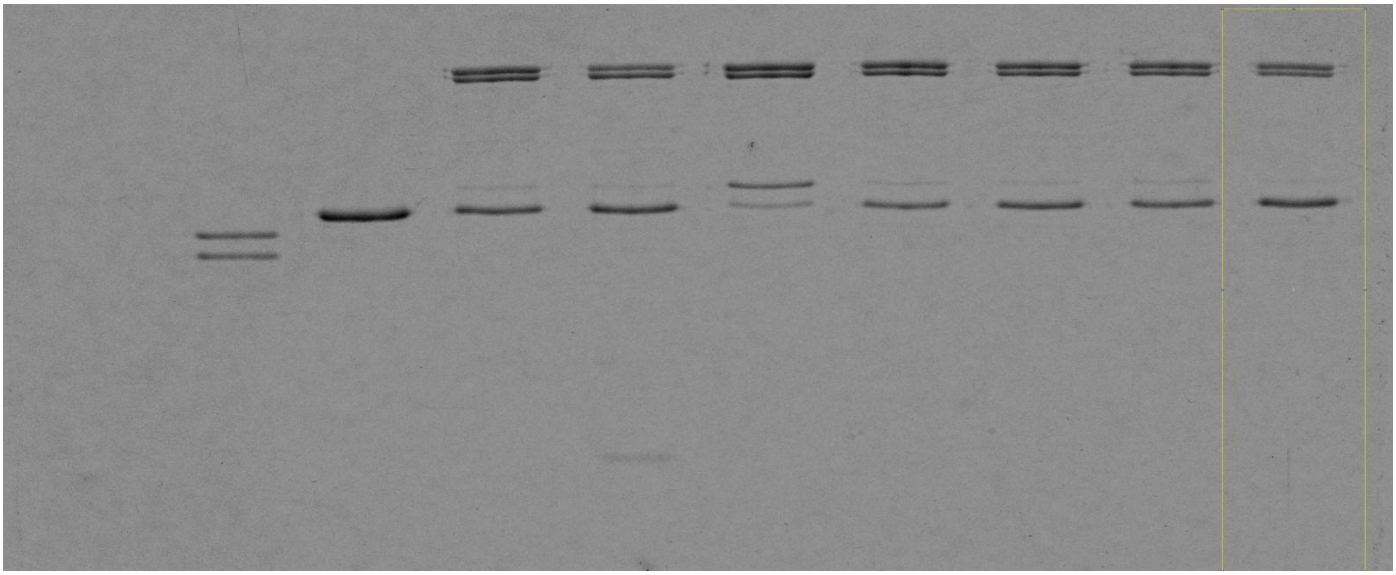
	1.62µg IXa and 4.10µg ATIII	*0.5µg SPD*

Mean	2.2655	0.7245
Variance	0.0022445	0.1235045
t Stat	7.16744186	
P(T<=t) one-tail	0.044125687	
t Critical one-tail	6.313751514	
P(T<=t) two-tail	0.088251374	
t Critical two-tail	12.70620473	

PS- inhibits formation of the IXa-ATIII complex and the SPM and SPD both inhibit the same complex but to a lesser extent. H, promotes formation of the complex.

Xa

The Effects of Heparin, Protamine Sulfate, Spermine, and Spermidine on the Interaction between Factor Xa and Anti-Thrombin III



1. 5µL Molecular Weight Markers+ 13µL Tris/NaCl pH8 + 5µL 5X Sample Buffer
2. 2.0µL (1.75µg) FXa + 13µL Tris/NaCl pH8 + 5µL 5X Sample Buffer
3. 2.0µL (4.10µg) ATIII + 13µL Tris/NaCl pH8 + 5µL 5X Sample Buffer
4. 2.0µL (1.75µg) FXa + 2.0µL (4.10µg) ATIII + 11µL Tris/NaCl pH8 + 5µL 5X Sample Buffer
5. [(2.0µL (4.10µg) ATIII + 1µg (3µL) Protamine Sulfate) 15’ at RT, + 2.0µL (1.75µg) FXa + 8µL Tris/NaCl pH8] 60’ at RT, + 5µL 5X Sample Buffer]
6. [(2.0µL (4.10µg) ATIII + 1µg (3µL) Heparin) 15’ at RT, + 1.0µL (1.75µg) FXa + 8µL Tris/NaCl pH8] 60’ at RT, + 5µL 5X Sample Buffer]
7. [(2.0µL (4.10µg) ATIII + 0.07µg (3µL) Spermine) 15’ at RT, + 2.0µL (1.75µg) FXa + 8µL Tris/NaCl pH8] 60’ at RT, + 5µL 5X Sample Buffer]
8. [(2.0µL (4.10µg) ATIII + 0.7µg (3µL) Spermine) 15’ at RT, + 2.0µL (1.75µg) FXa + 8µL Tris/NaCl pH8] 60’ at RT, + 5µL 5X Sample Buffer]
9. [(2.0µL (4.10µg) ATIII + 0.05µg (3µL) Spermidine) 15’ at RT, + 2.0µL (1.75µg) FXa + 8µL Tris/NaCl pH8] 60’ at RT, + 5µL 5X Sample Buffer]
10. [(2.0µL (4.10µg) ATIII + 0.5µg (3µL) Spermidine) 15’ at RT, + 2.0µL (1.75µg) FXa + 8µL Tris/NaCl pH8] 60’ at RT, + 5µL 5X Sample Buffer]

Pictures of Each Gel:
Figure A

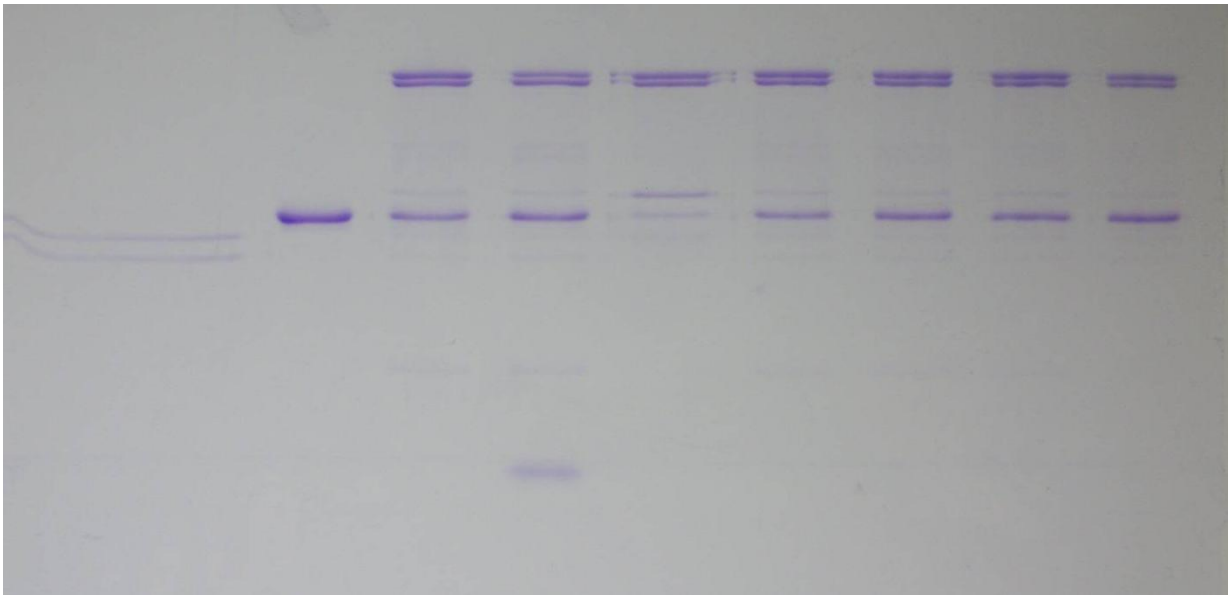
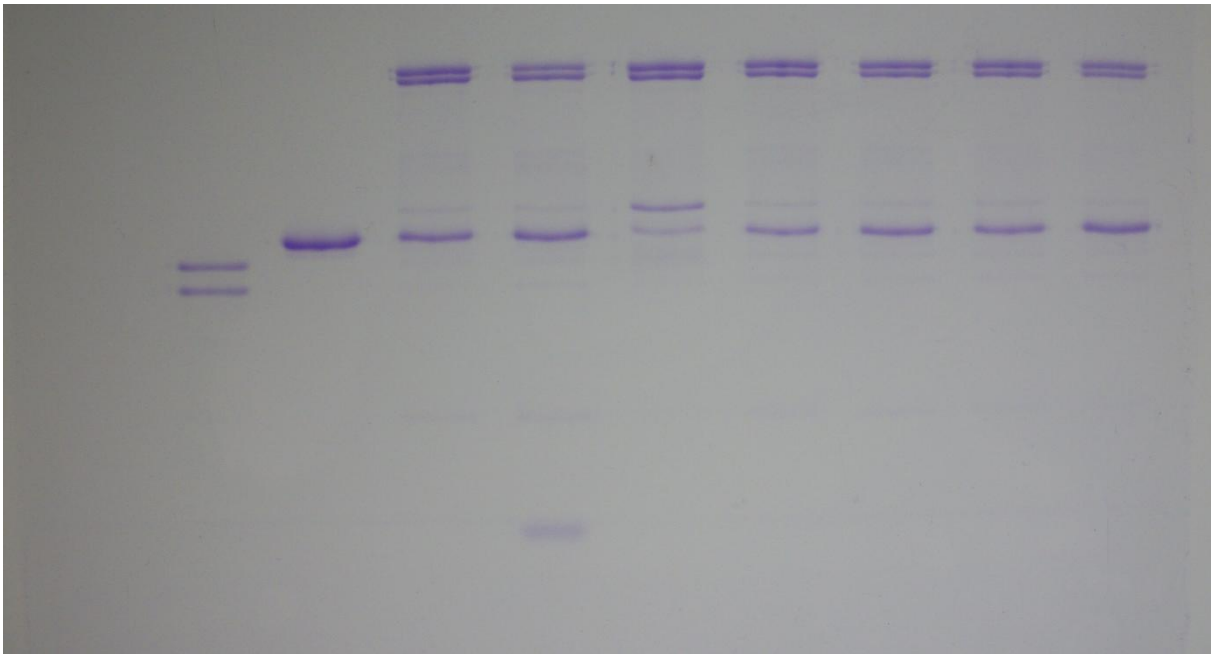


Figure B



Gel 1 will be analyzed first, followed by Gel 2, 3, and 4.

Table 1: Overall Protein Analysis Per Lane

*ND=Not Detected

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Primary Alpha	37.13	22.9	42.14	39.8	36.2	37.3	28.4
Primary Beta	28.75	27.4	32.63	29.1	25.9	28.1	21.1
Secondary Alpha	*ND	*ND	*ND	*ND	*ND	*ND	*ND
Secondary Beta	*ND	*ND	*ND	*ND	*ND	*ND	*ND
Tertiary Alpha	0.786	0.37	0.357	0.459	0.36	0.647	0.664
Tertiary Beta	1.05	0.646	0.125	0.615	0.693	0.479	0.414
ATIIIM	1.92	1.88	15.65	2.861	1.66	2.39	3.59
ATIII	29	35.28	7.84	25.6	34	30.01	44.04
Xa Alpha	0.832	0.986	0.87	0.66	0.471	0.469	1.53
Xa Beta	0.535	1.364	0.396	0.937	0.702	0.663	0.277
Solvent Front	*ND	*ND	*ND	*ND	*ND	*ND	*ND
PS Degradation	*ND	9.1	*ND	*ND	*ND	*ND	*ND

Standard Deviation	15.08682252	Mean	12.281193	Standard Error	1.777999

Figure C: Graphical Representation of Table 1

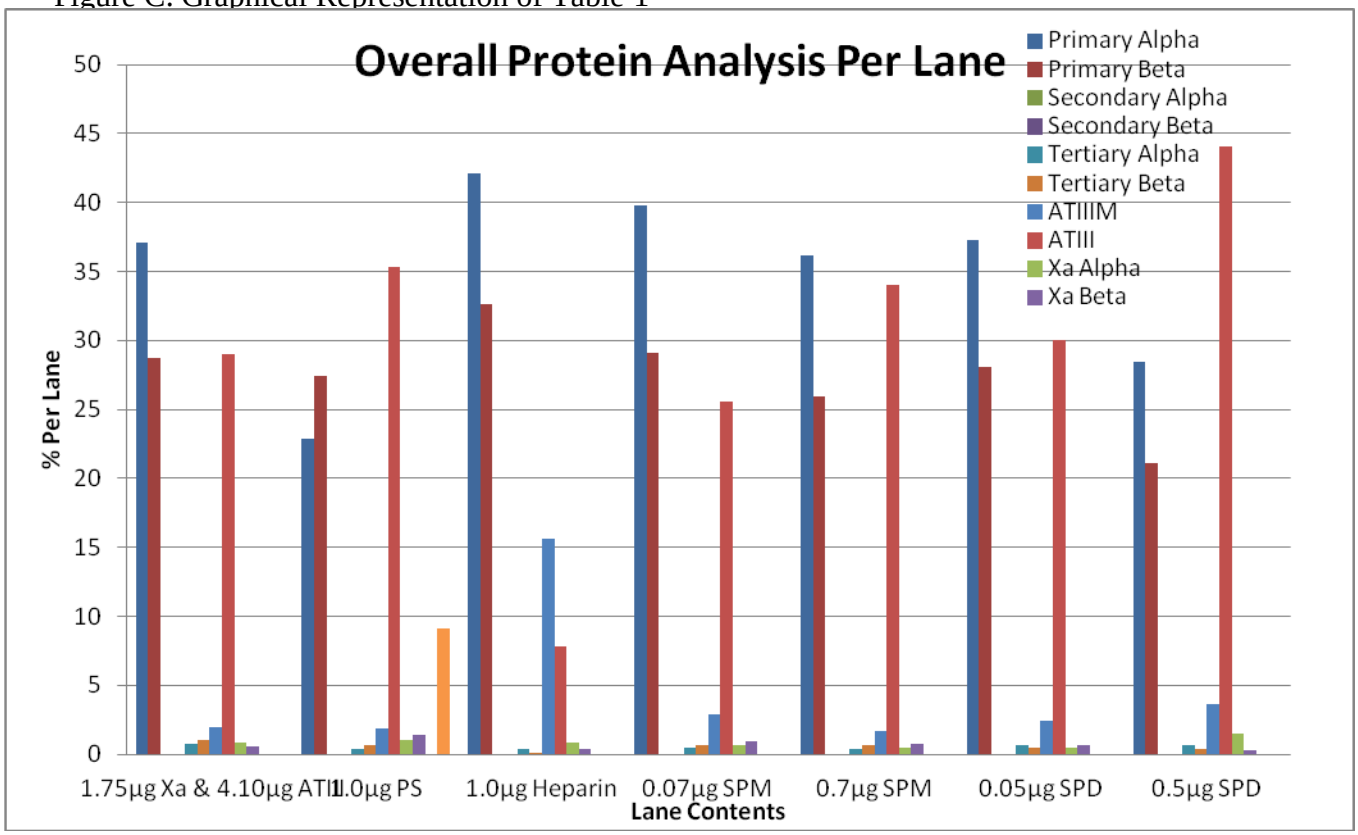


Table 2: Primary Alpha [Xa-ATIII] Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Primary Alpha	37.13	22.9	42.14	39.8	36.2	37.3	28.4

Figure D: The Graphical Representation of Table 2

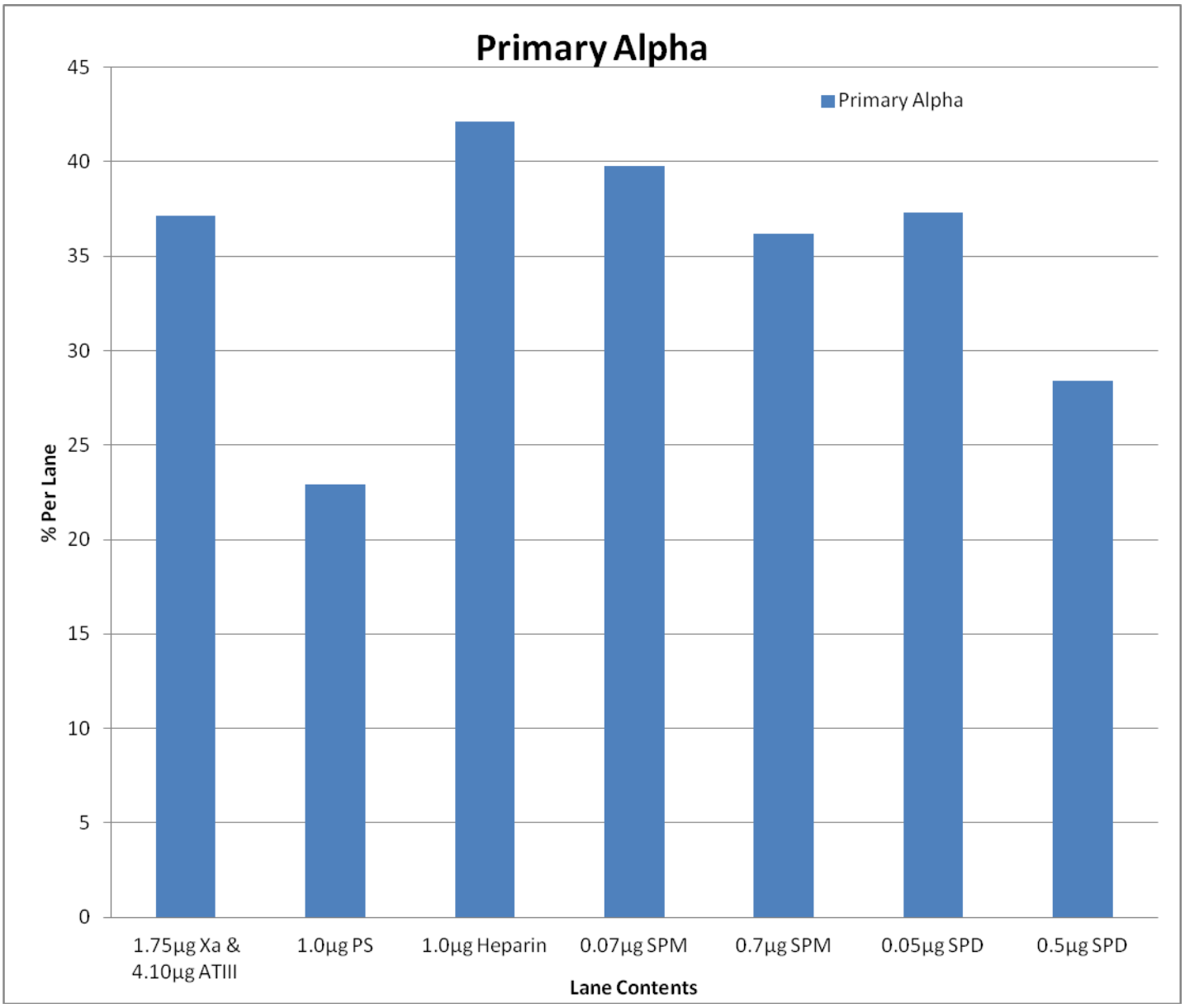


Table 3: Primary Beta [Xa-ATII] Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Primary Beta	28.75	27.4	32.63	29.1	25.9	28.1	21.1

Figure E: Graphical Representation of Table 3

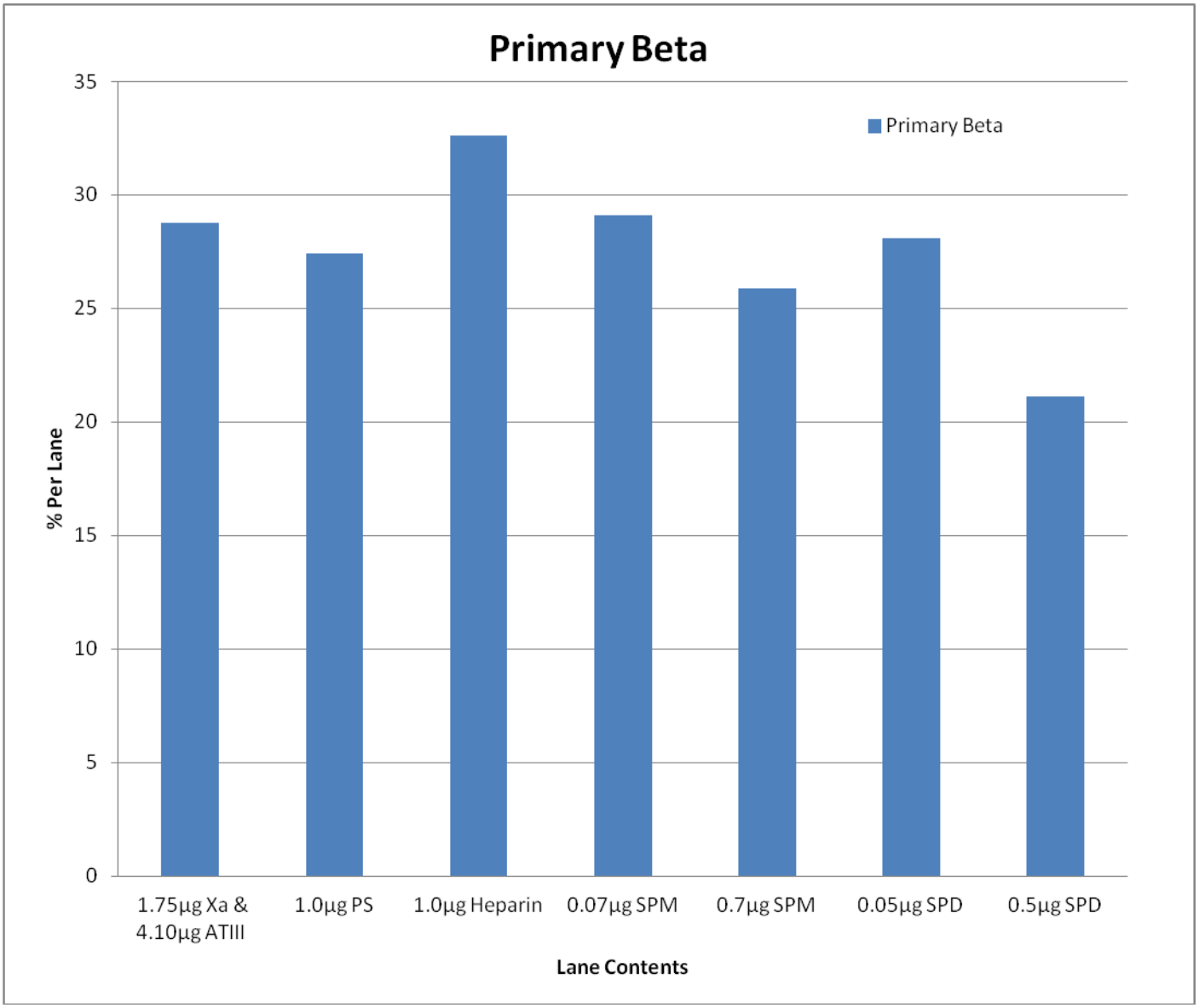


Table 4: Tertiary Alpha [Xa-ATIII] Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Tertiary Alpha	0.786	0.37	0.357	0.459	0.36	0.647	0.664

Figure F: Graphical Representation of Table 4

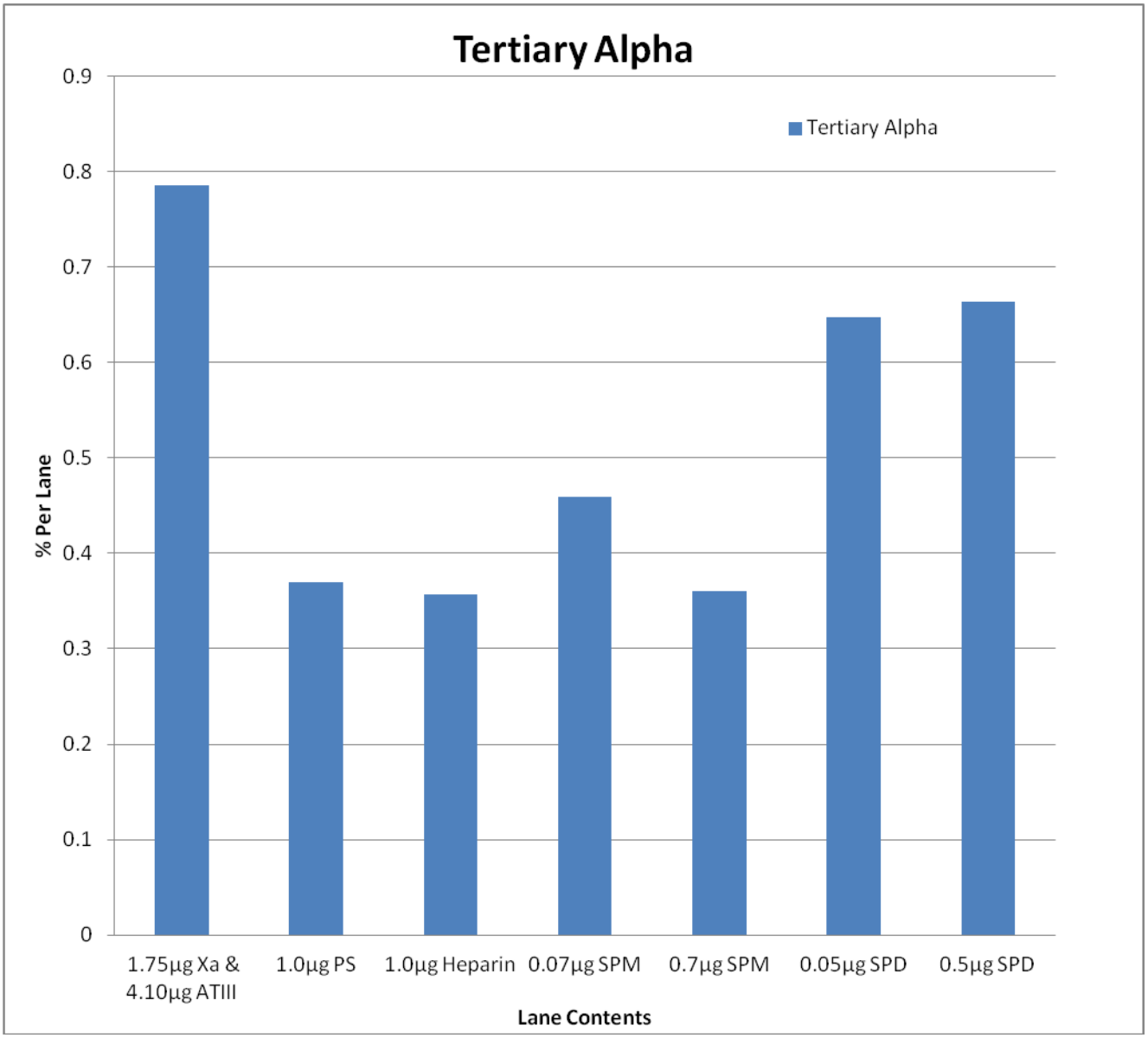


Table 5: Tertiary Beta [Xa-ATIII] Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Tertiary Beta	1.05	0.646	0.125	0.615	0.693	0.479	0.414

Figure G: Graphical Representation of Table 5

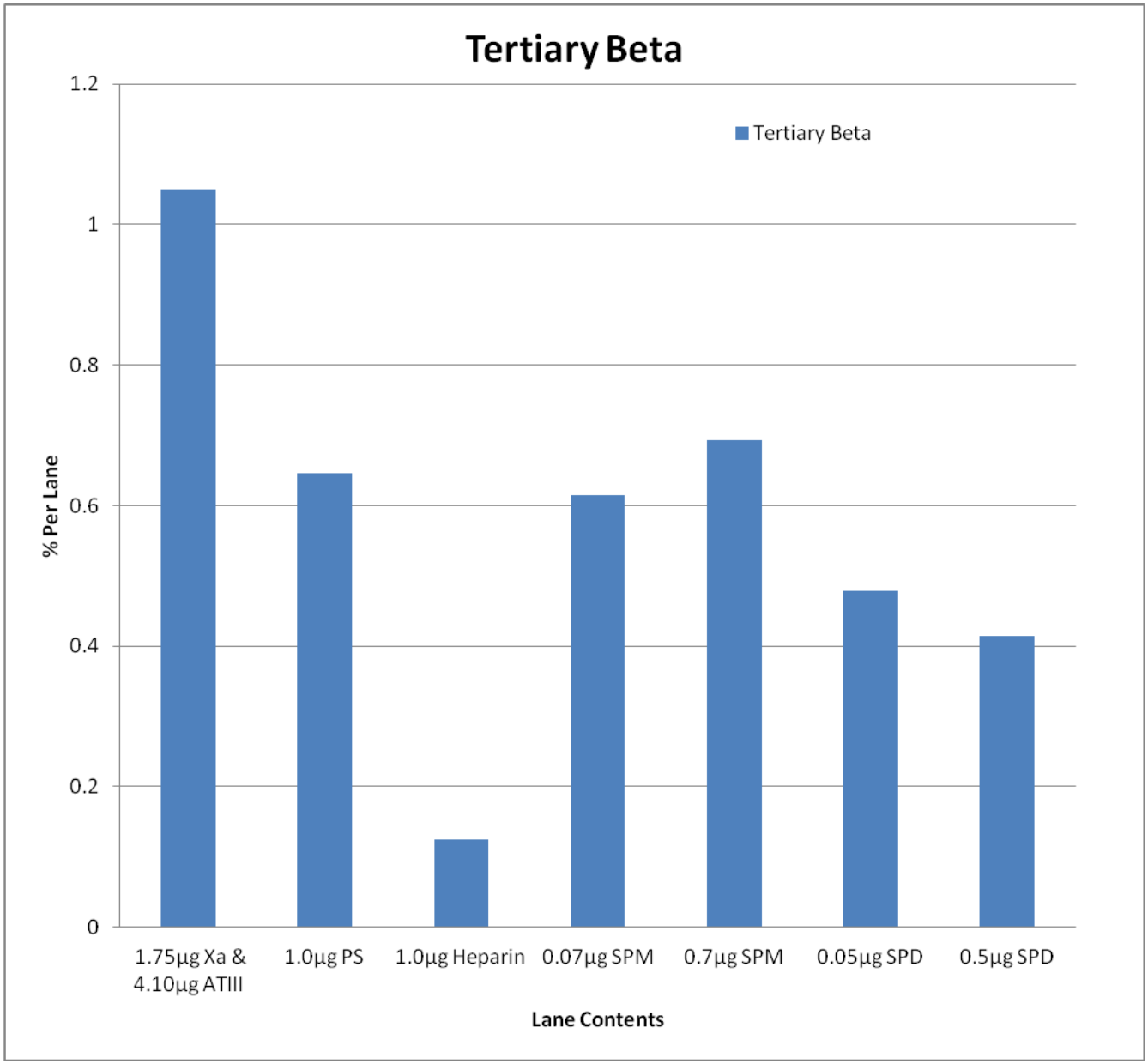


Table 6: ATIII-M Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIIIM	1.92	1.88	15.65	2.861	1.66	2.39	3.59

Figure H: Graphical Representation of Table 6

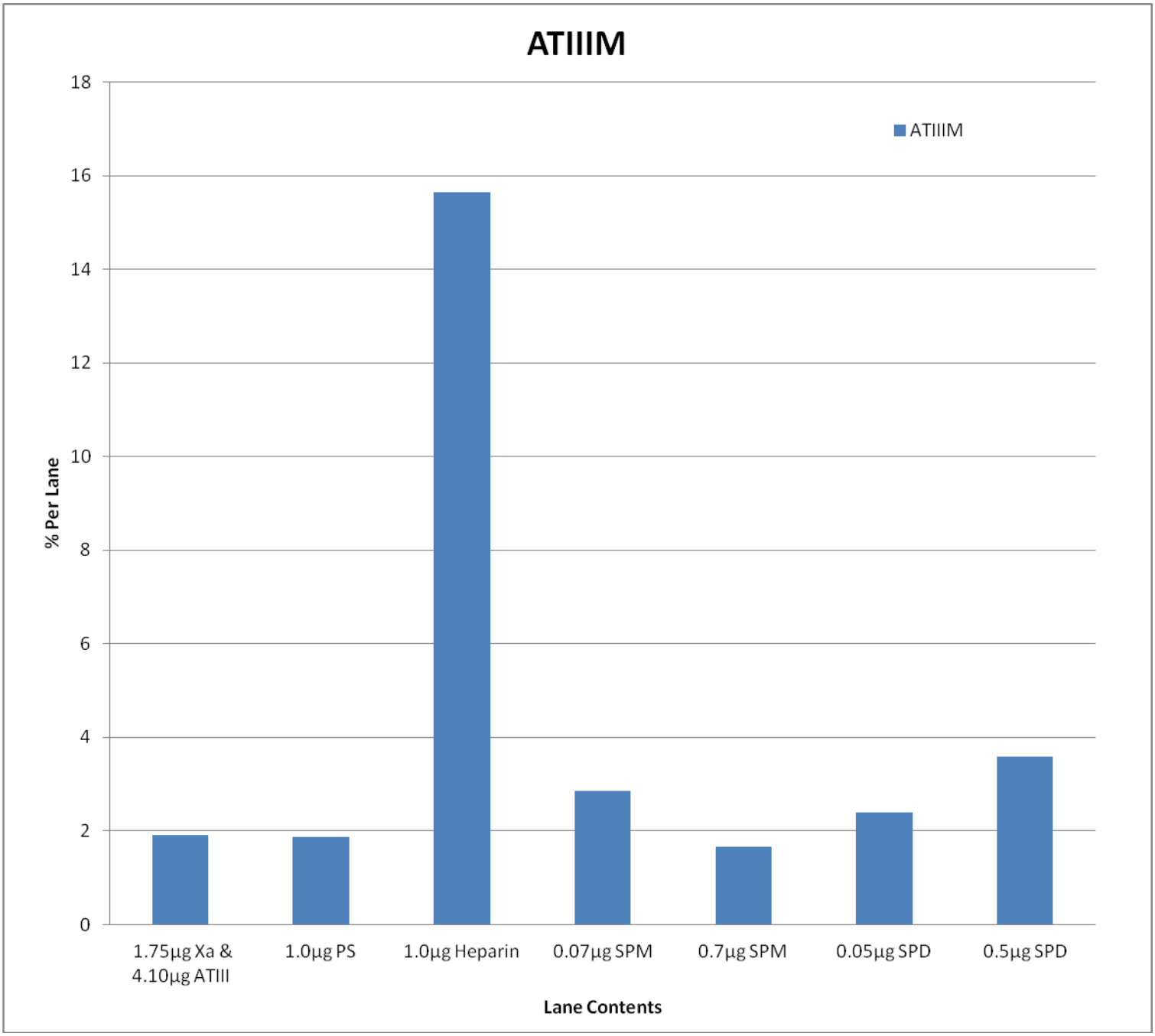


Table 7: ATIII Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIII	29	35.28	7.84	25.6	34	30.01	44.04

Figure I: Graphical Representation of Table 7

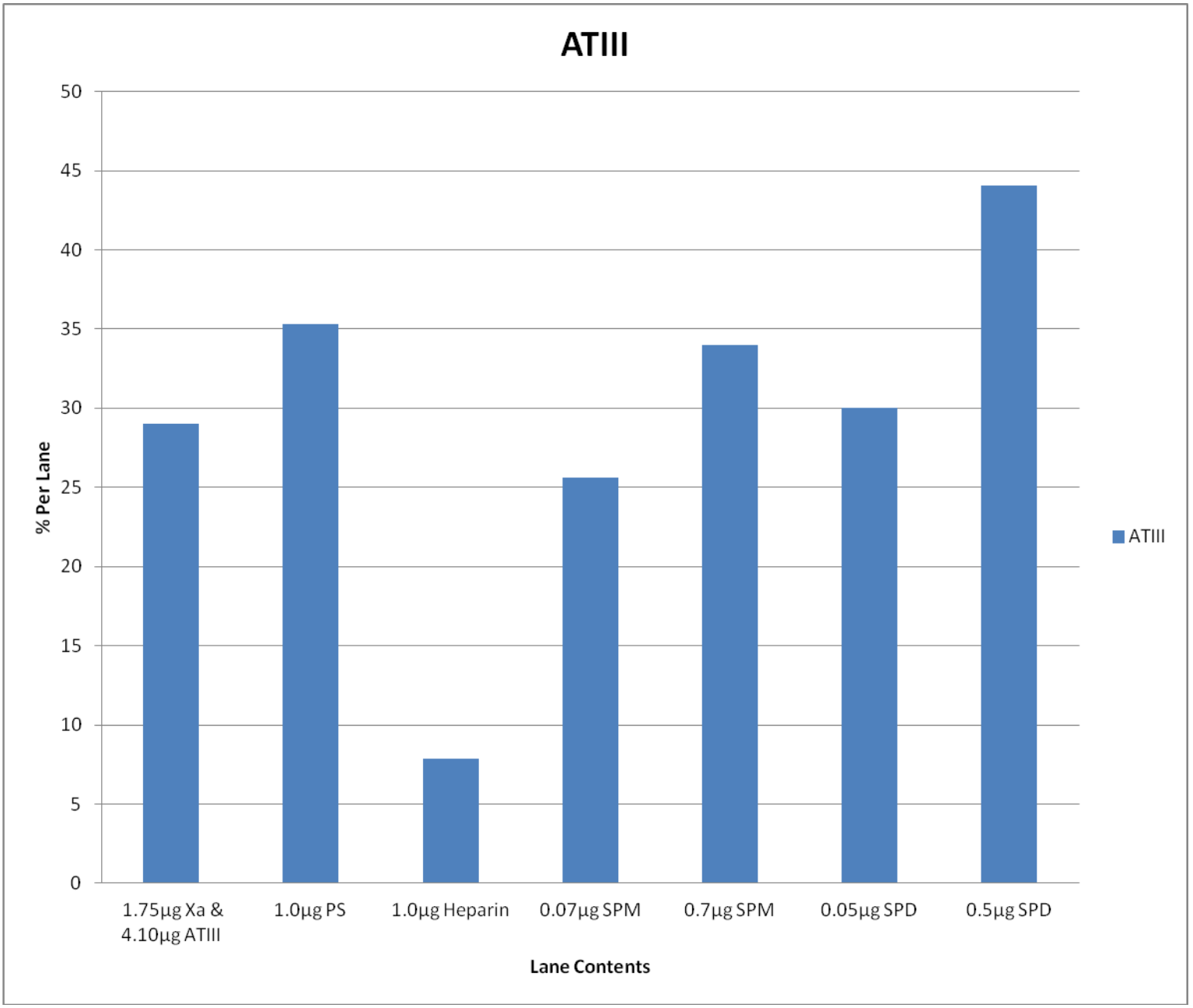


Table 8: Xa Alpha Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Xa Alpha	0.832	0.986	0.87	0.66	0.471	0.469	1.53

Figure J: Graphical Representation of Table 8

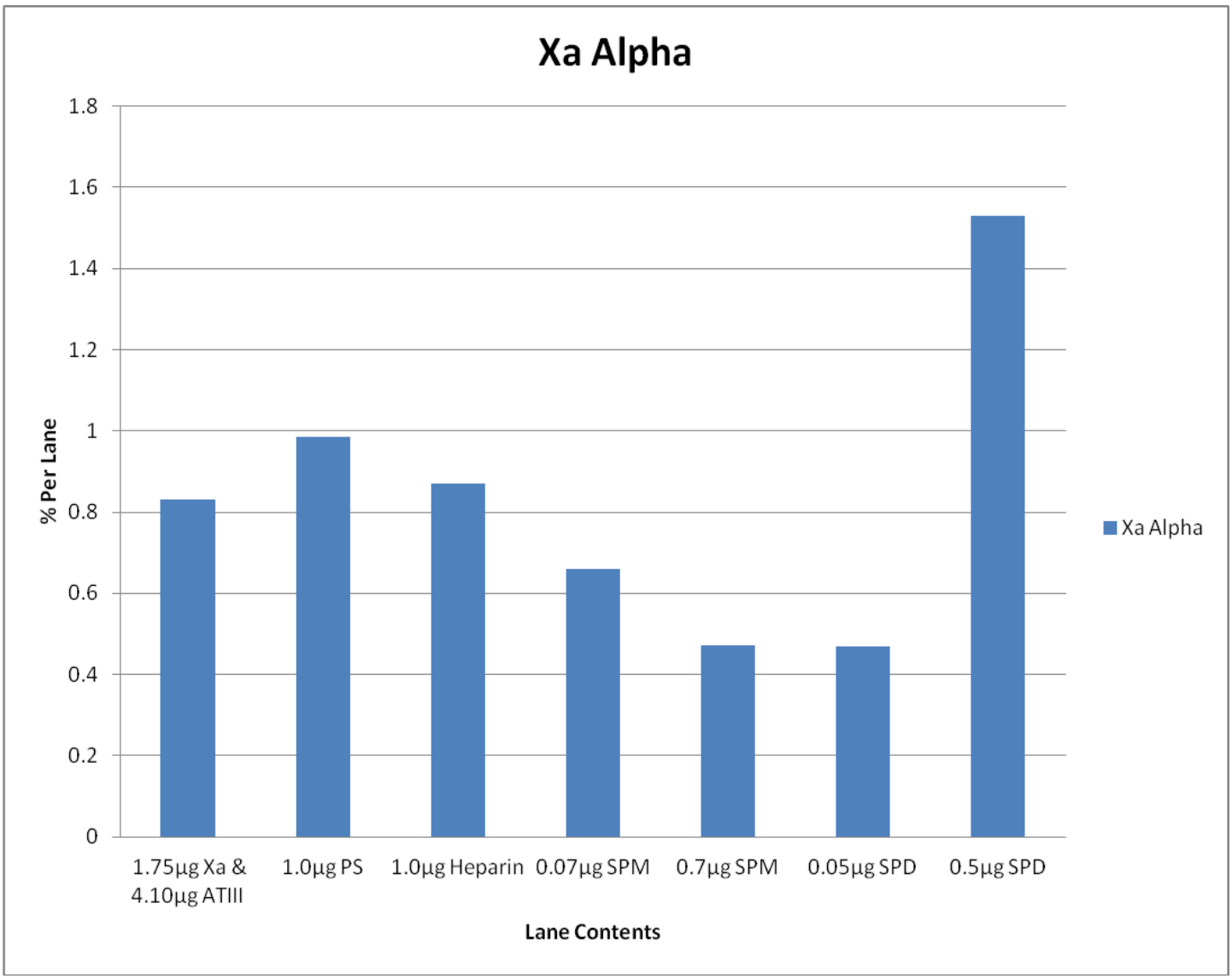


Table 9: Xa Beta Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Xa Beta	0.535	1.364	0.396	0.937	0.702	0.663	0.277

Figure K: Graphical Representation of Table 9

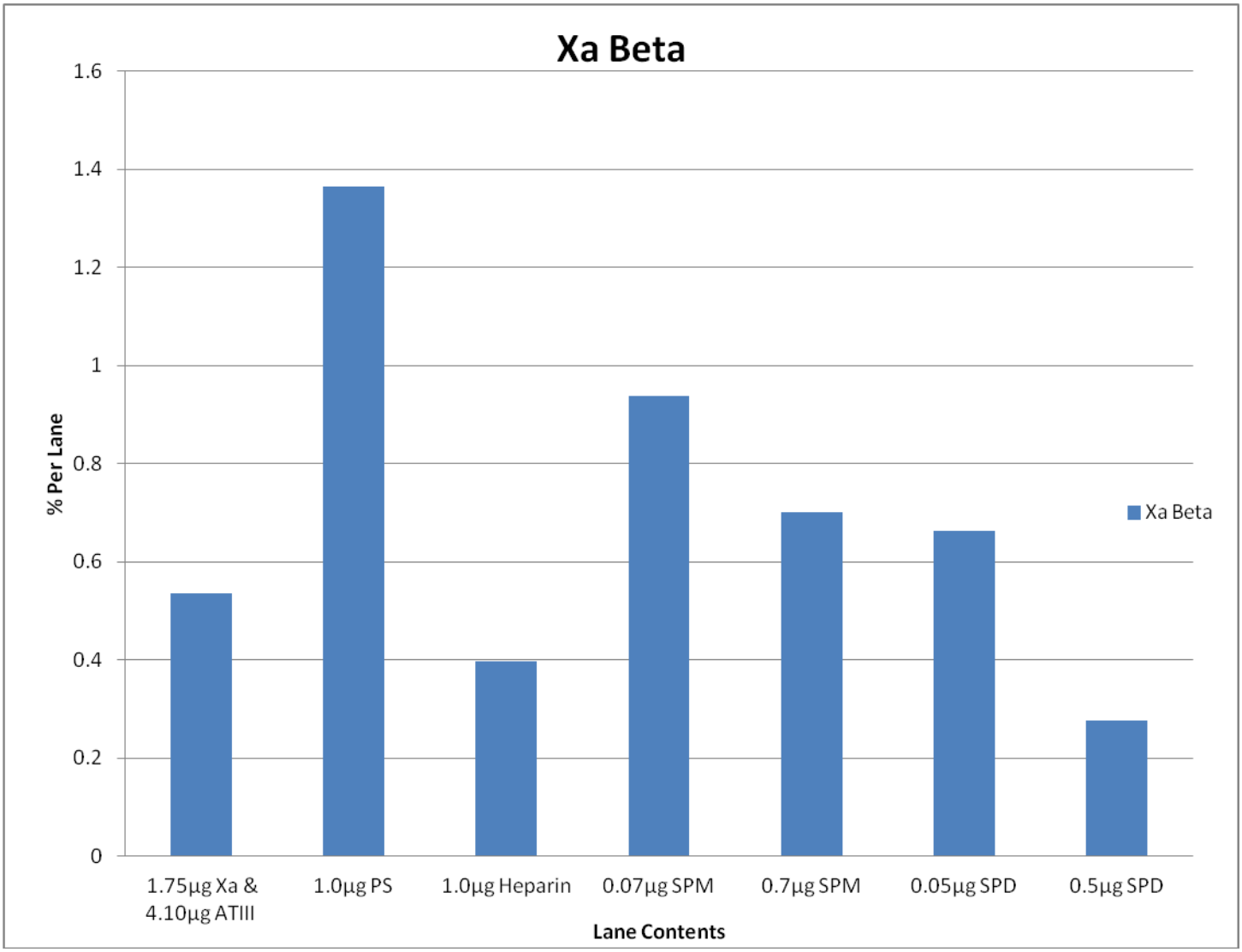
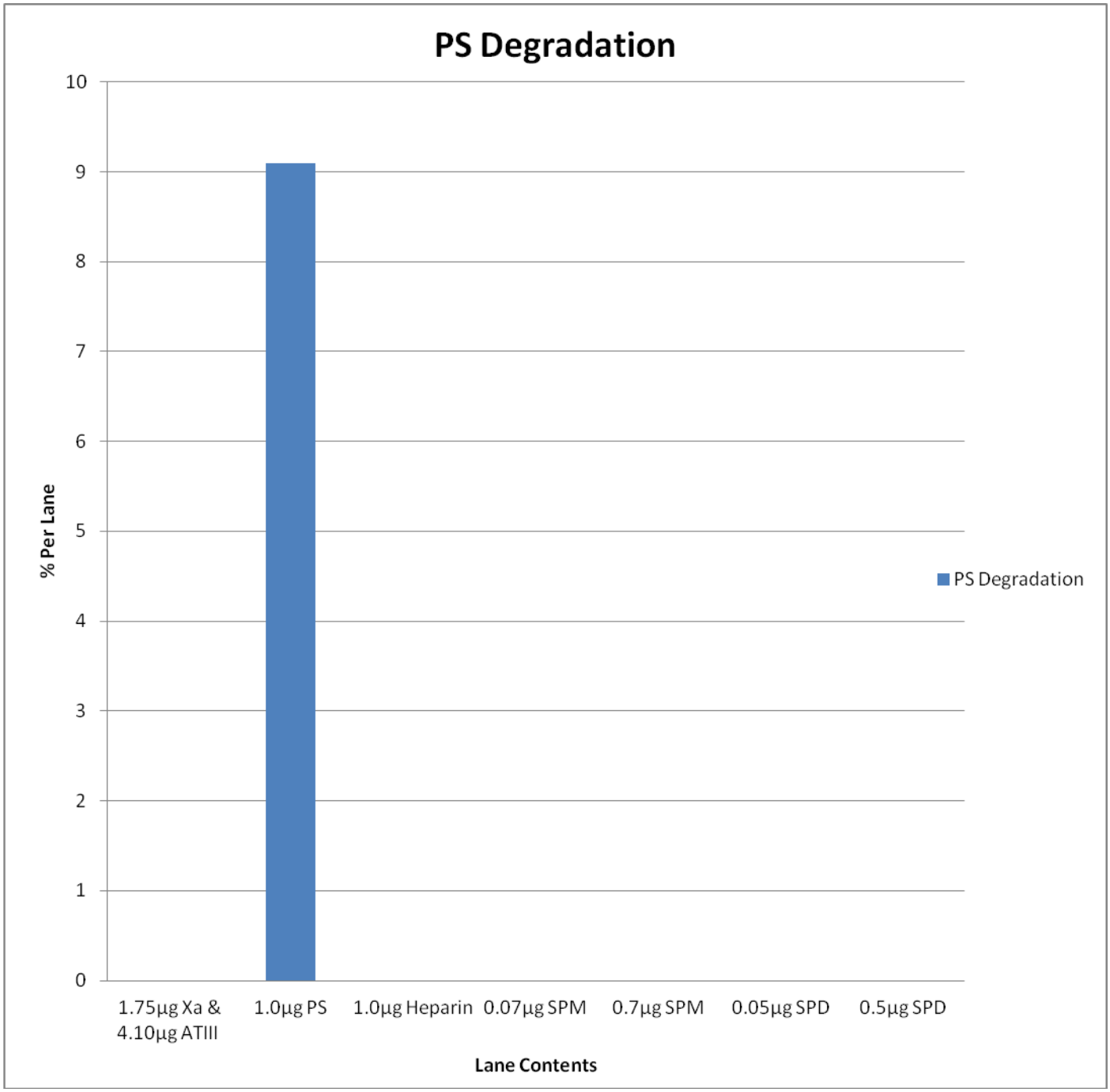


Table 10: PS Degradation Analysis Per Lane
*ND= Not Detected

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
PS Degradation	*ND	9.1	*ND	*ND	*ND	*ND	*ND

Figure L: Graphical Representation of Table 10



Gel 2

Table 11: Overall Protein Analysis Per Lane
*ND= Not Detected

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Primary Alpha	36.2	24	39.2	35.8	33.7	37.4	29.4
Primary Beta	28.1	26.9	32.7	30.5	28.6	26.9	22
Secondary Alpha	*ND	*ND	*ND	*ND	*ND	*ND	*ND
Seconday Beta	*ND	*ND	*ND	*ND	*ND	*ND	*ND
Tertiary Alpha	1.34	1.225	0.963	1.38	1.72	0.539	1.18
Tertiary Beta	1.32	0.452	0.954	2.25	1.22	1.16	0.72
ATIIIM	2.06	2.46	17.7	2.12	0.24	2.98	1.39
ATIII	30	36.9	7.3	23.3	31.6	29.7	42.5
Xa Alpha	0.3456	0.598	0.88	1.75	0.974	0.493	2.15
Xa Beta	0.697	0.912	0.373	2.98	0.971	0.88	0.781
Solvent Front	*ND	1.27	*ND	*ND	*ND	*ND	*ND
PS Degradation	*ND	5.24	*ND	*ND	*ND	*ND	*ND

Standard Deviation	14.60976948	Mean	12.0580621	Standard Error	1.721778

Figure M: Graphical Representation of Table 11

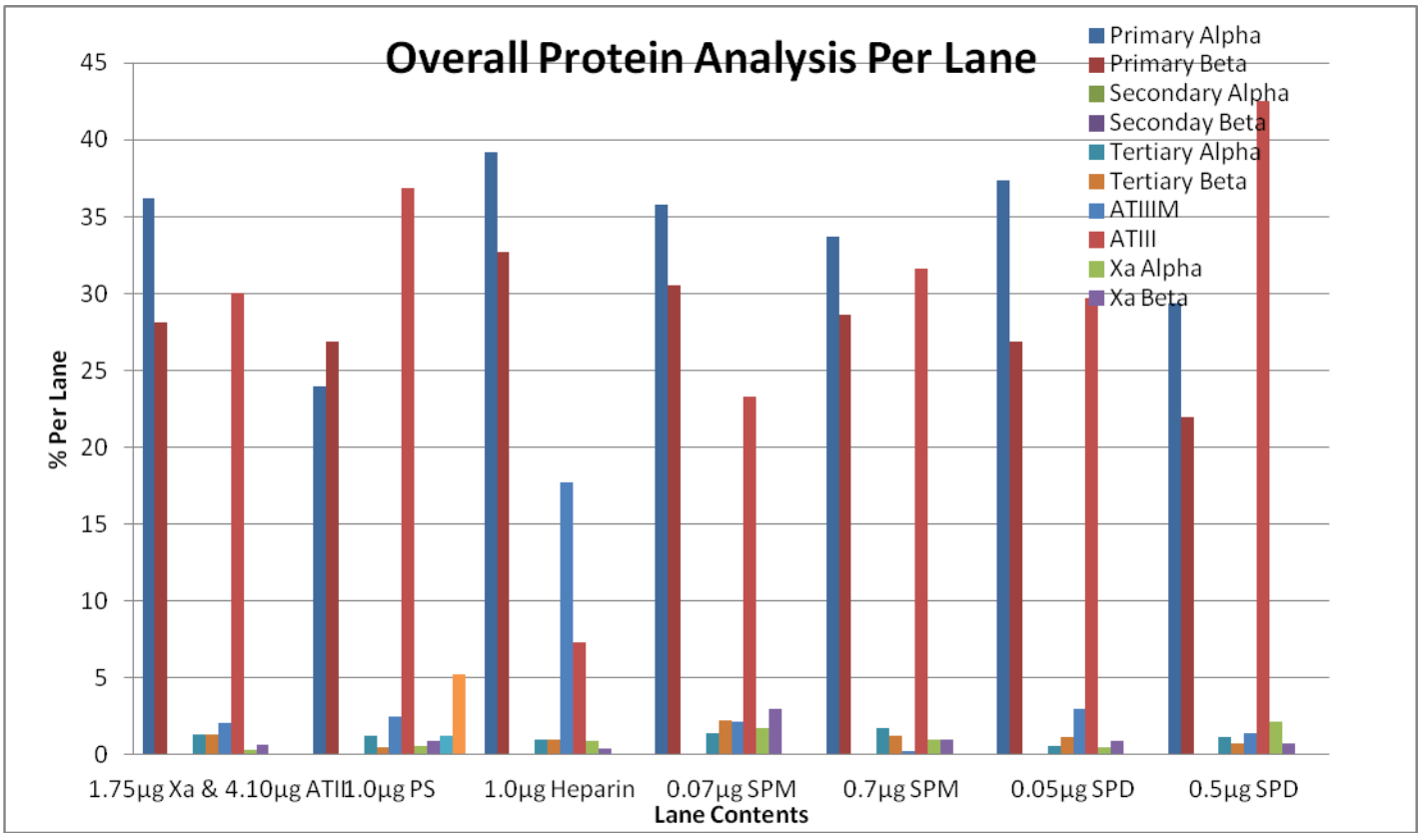


Table 12: Primary Alpha [Xa-ATIII] Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Primary Alpha	36.2	24	39.2	35.8	33.7	37.4	29.4

Figure N: Graphical Representation of Table 12

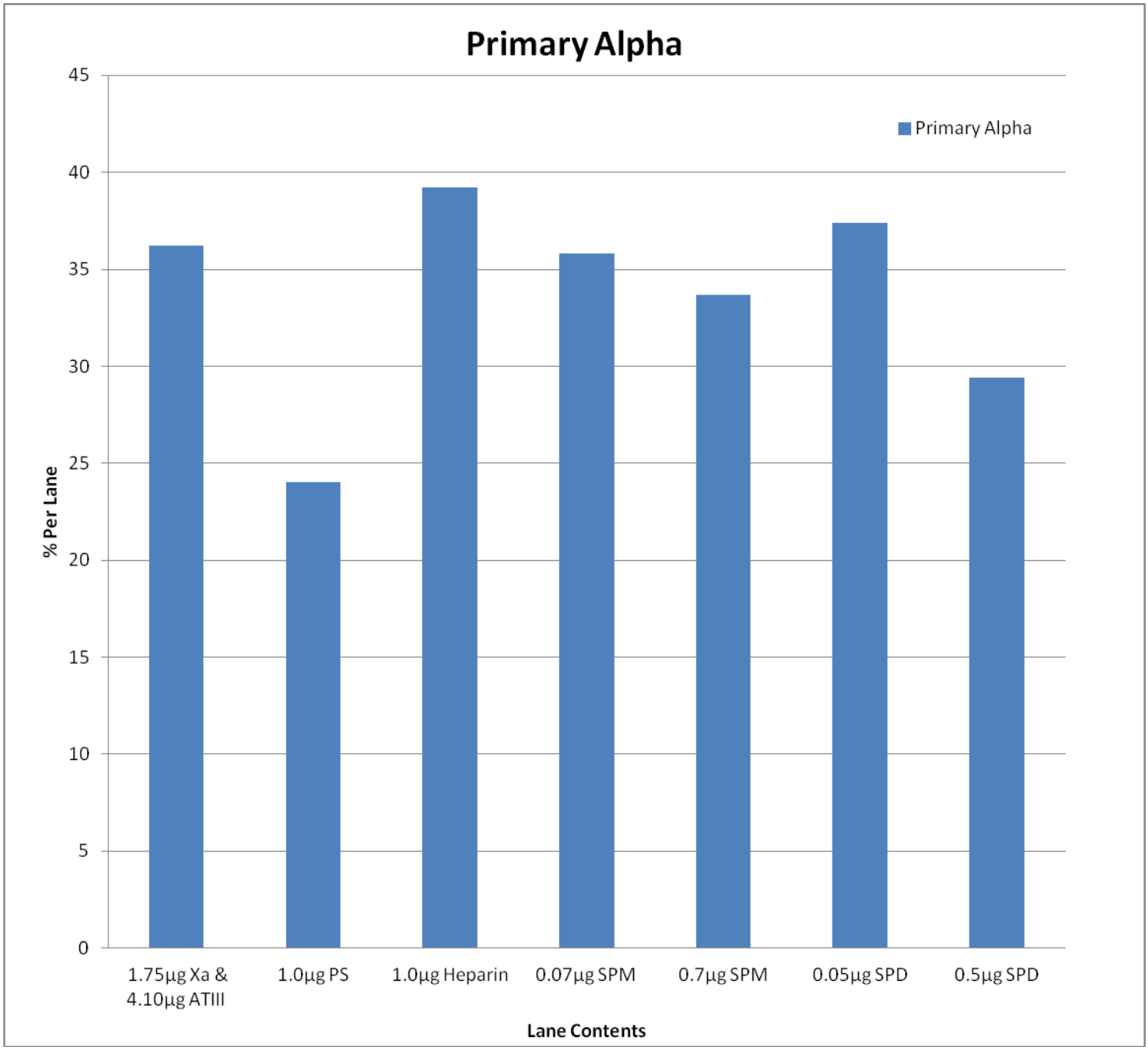


Table 13: Primary Beta [Xa-ATIII] Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Primary Beta	28.1	26.9	32.7	30.5	28.6	26.9	22

Figure O: Graphical Representations of Table 13

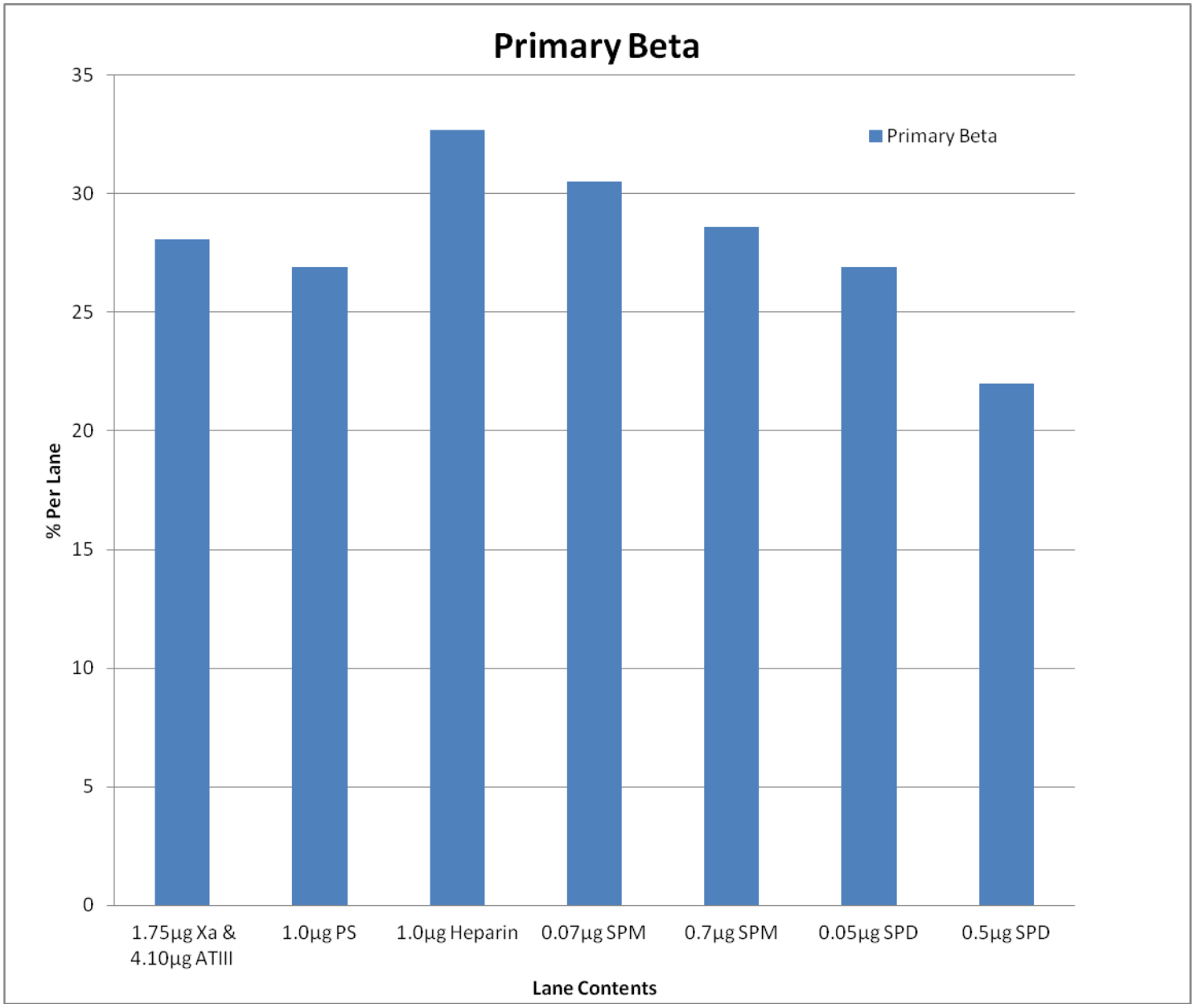


Table 14: Tertiary Alpha [Xa-ATIII] Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Tertiary Alpha	1.34	1.225	0.963	1.38	1.72	0.539	1.18

Figure P: Graphical Representation of Table 14

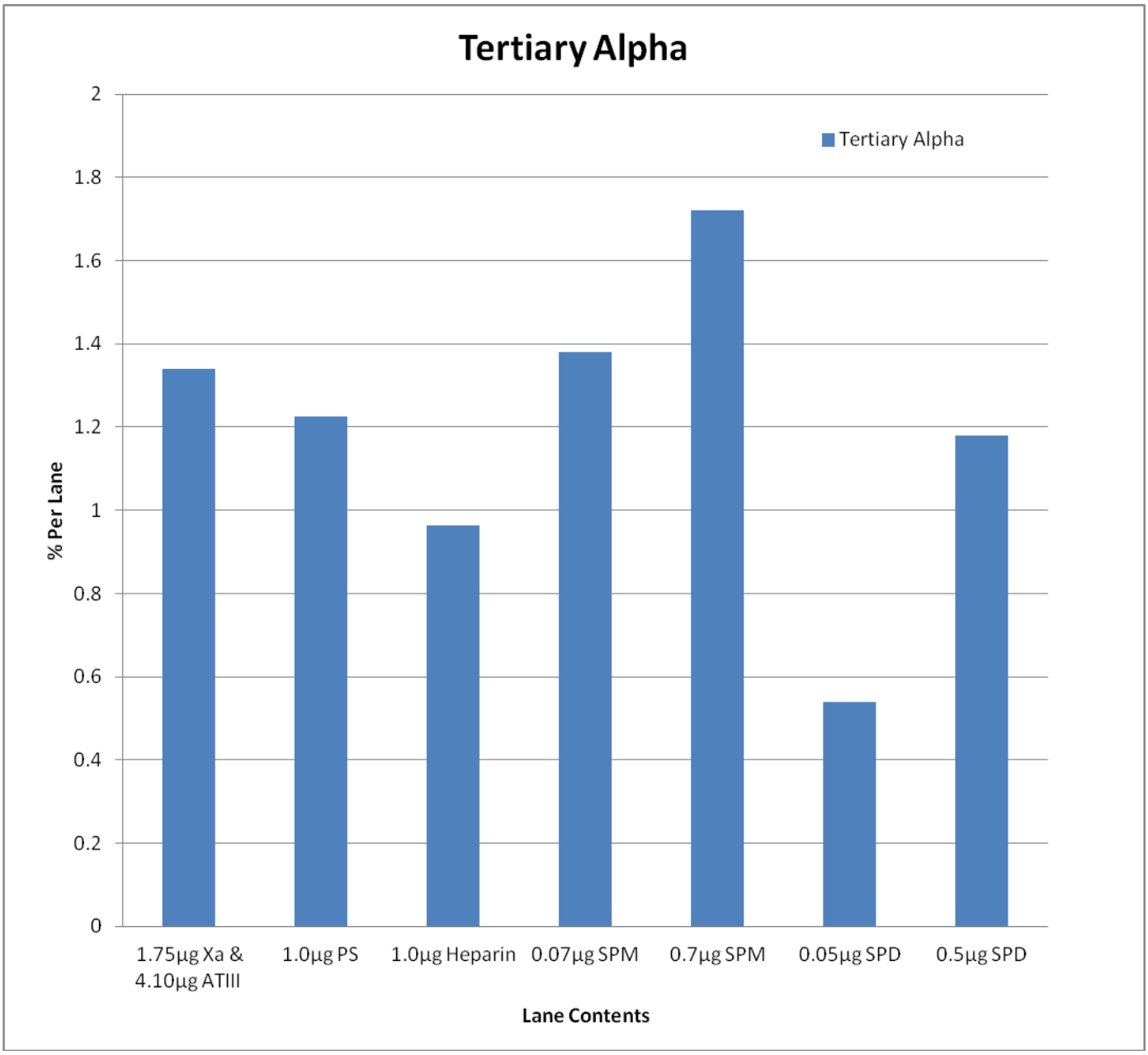


Table 15: Tertiary Beta [Xa-ATIII] Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Tertiary Beta	1.32	0.452	0.954	2.25	1.22	1.16	0.72

Figure Q: Graphical Representation of Table 15

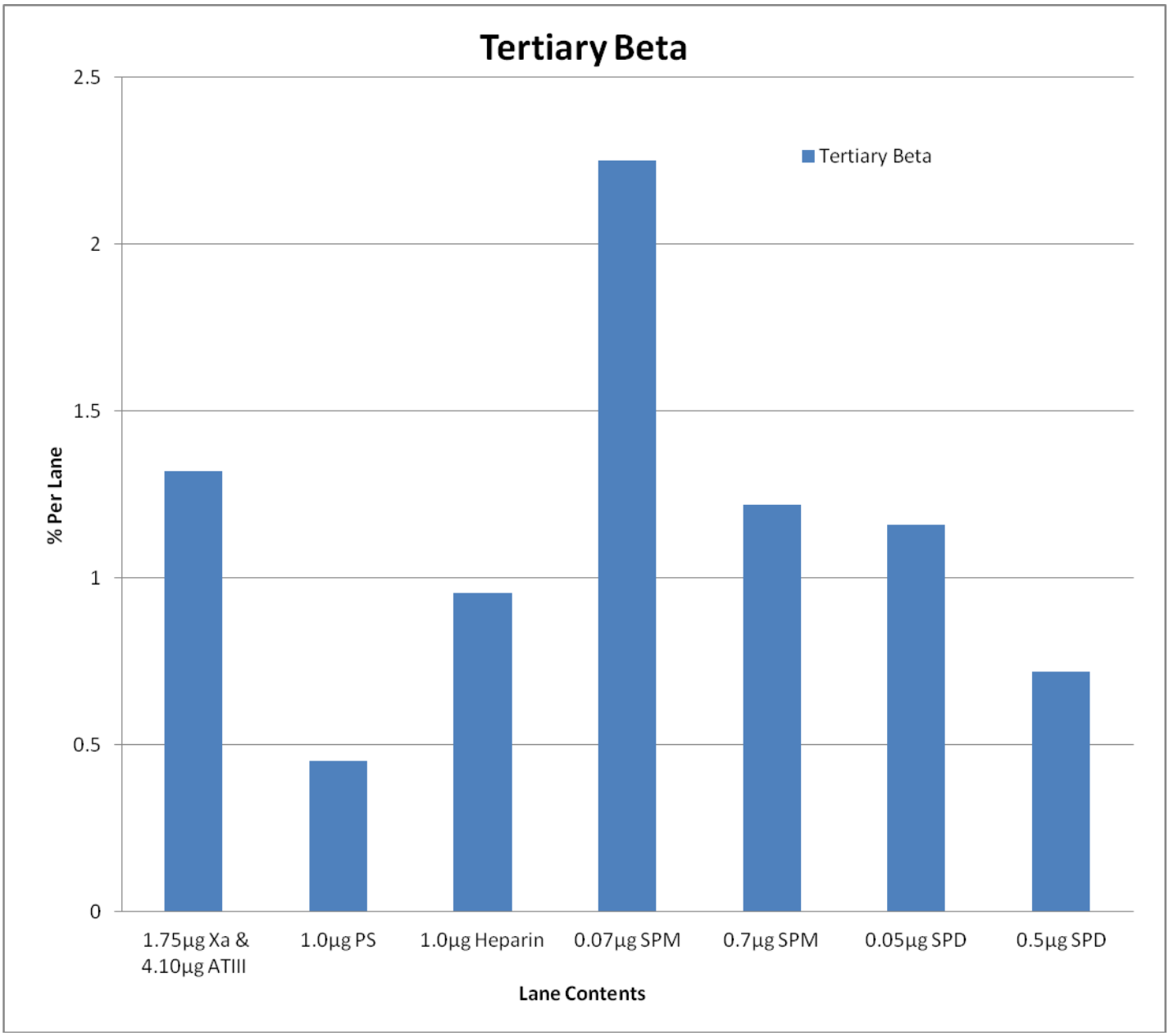


Table 16: ATIII-M Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIIIM	2.06	2.46	17.7	2.12	0.24	2.98	1.39

Figure R: Graphical Representation of Table 16

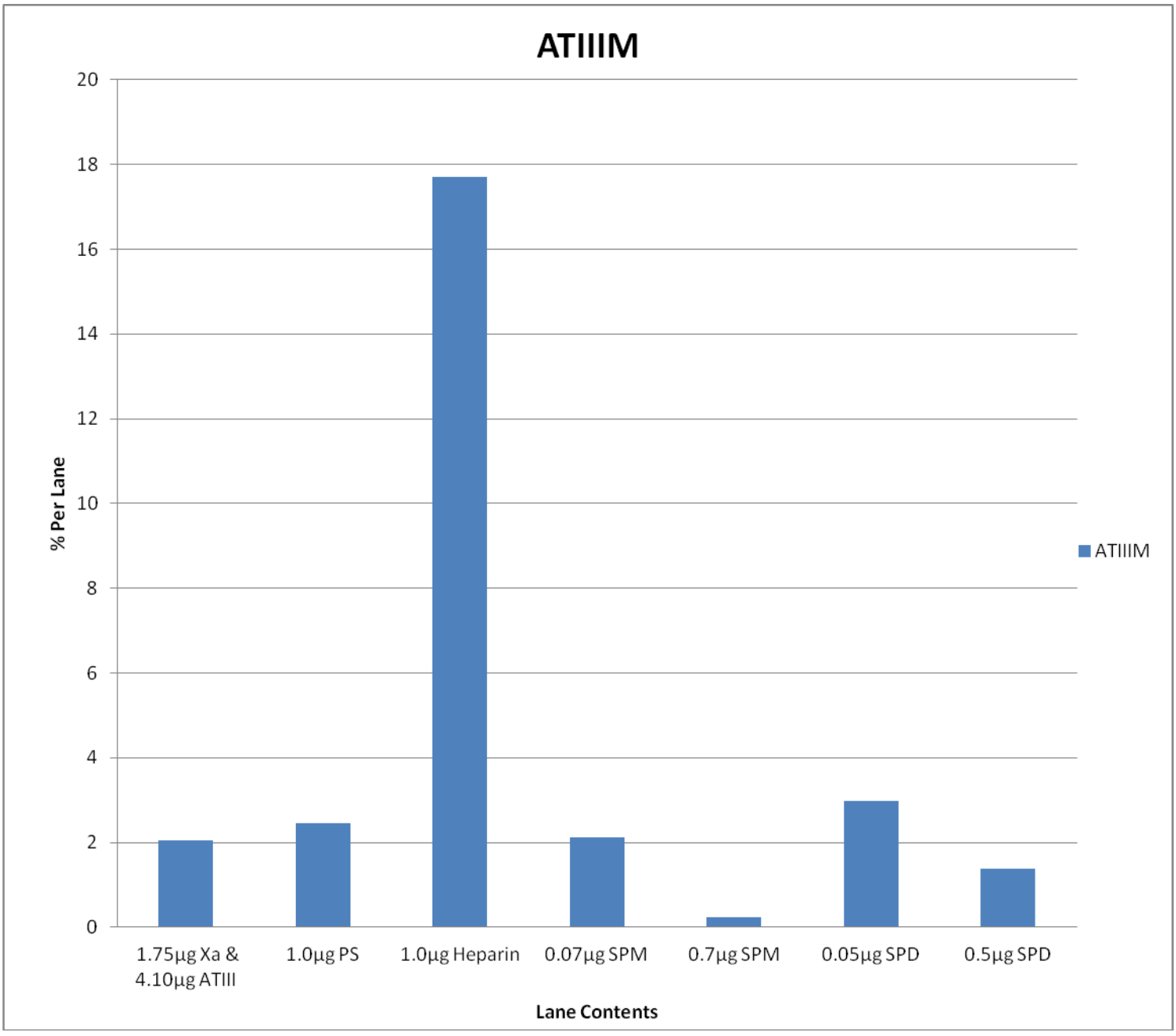


Table 17: ATIII Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIII	30	36.9	7.3	23.3	31.6	29.7	42.5

Figure S: Graphical Representation of Table 17

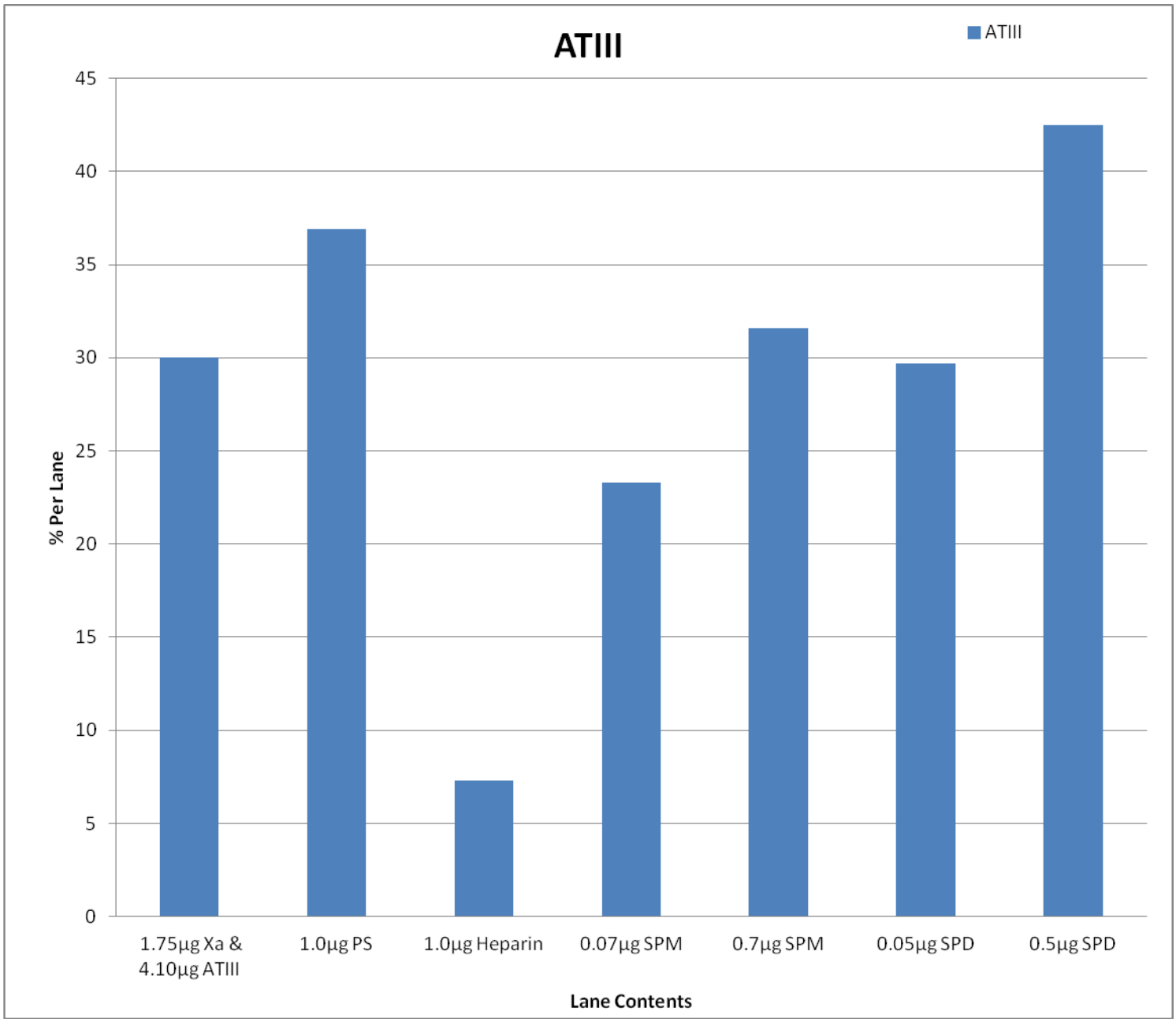


Table 18: Xa Alpha Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Xa Alpha	0.3456	0.598	0.88	1.75	0.974	0.493	2.15

Figure T: Graphical Representation of Table 18

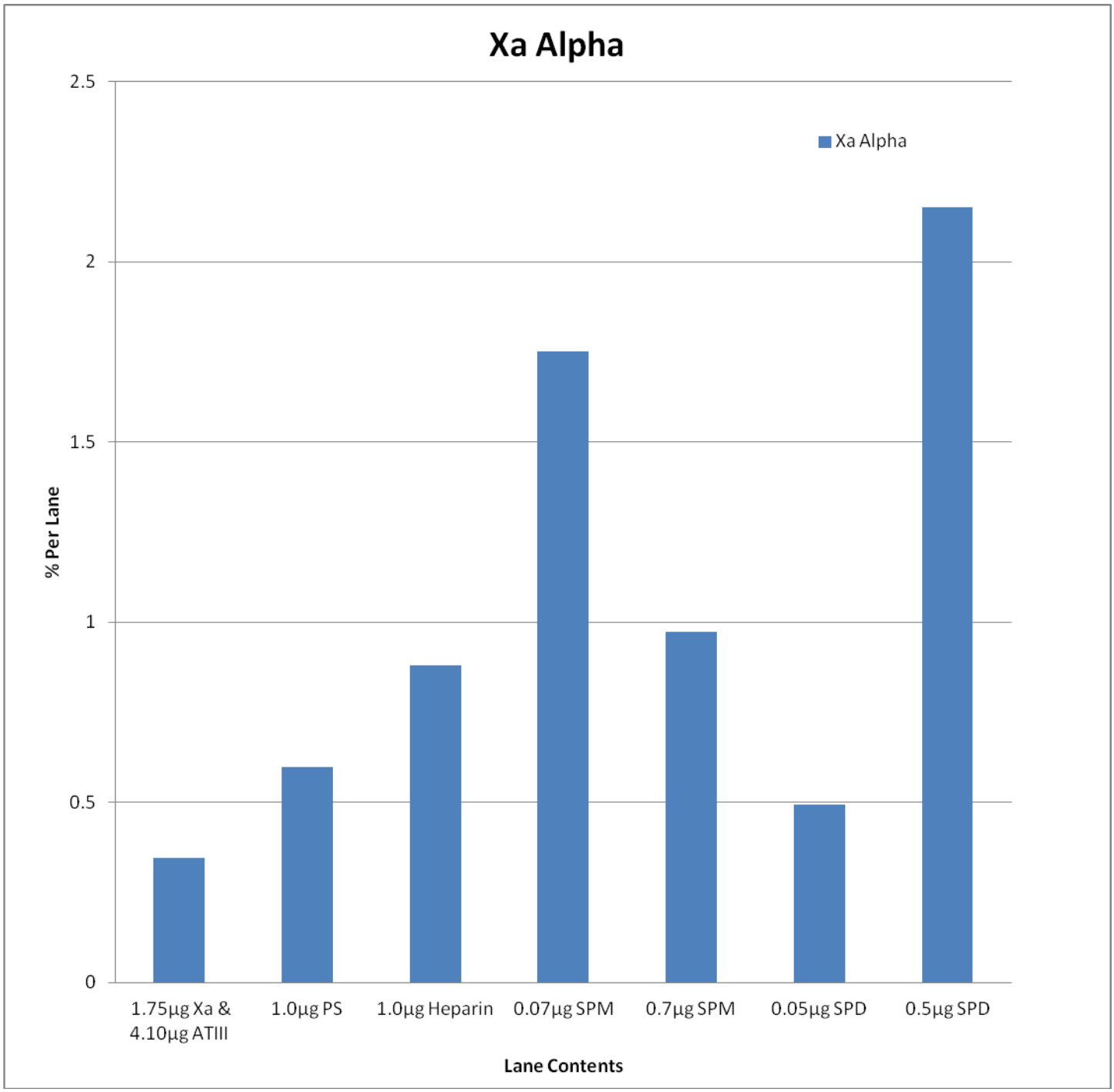


Table 19: Xa Beta Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Xa Beta	0.697	0.912	0.373	2.98	0.971	0.88	0.781

Figure U: Graphical Representation of Table 19

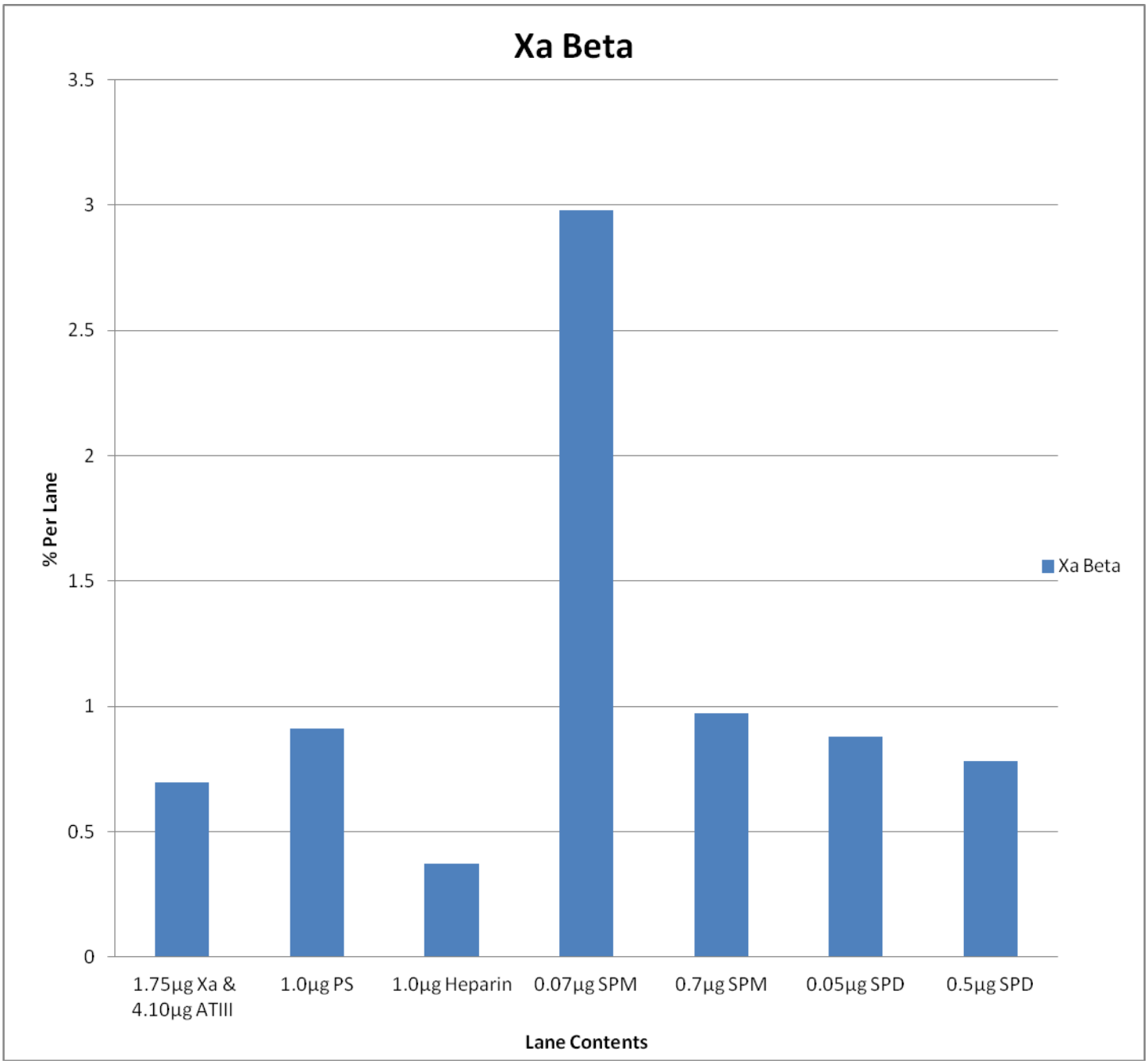


Table 20: Solvent Front Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Solvent Front	*ND	1.27	*ND	*ND	*ND	*ND	*ND

*ND= Not Detected

Figure V: Graphical Representation of Table 20

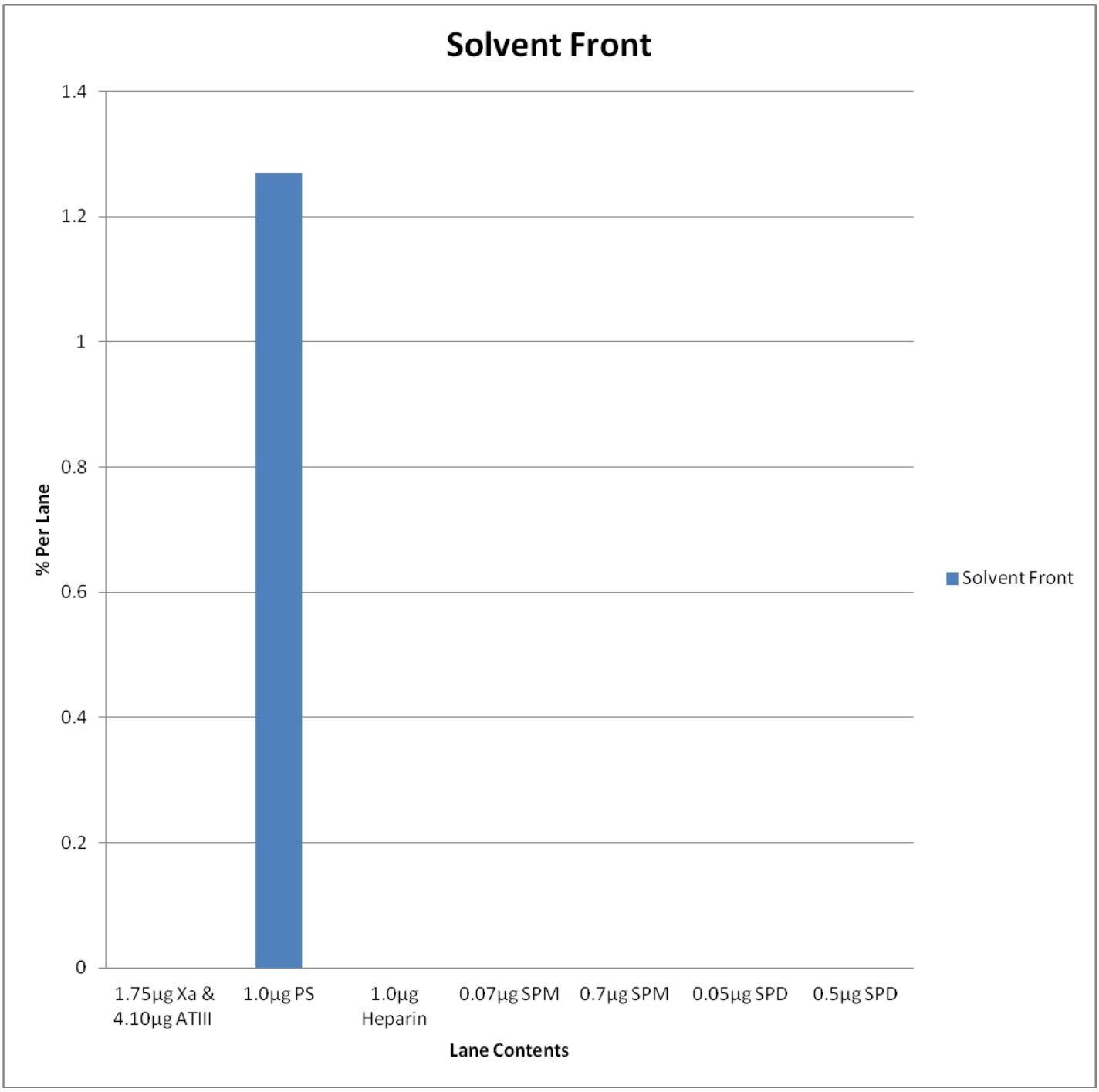
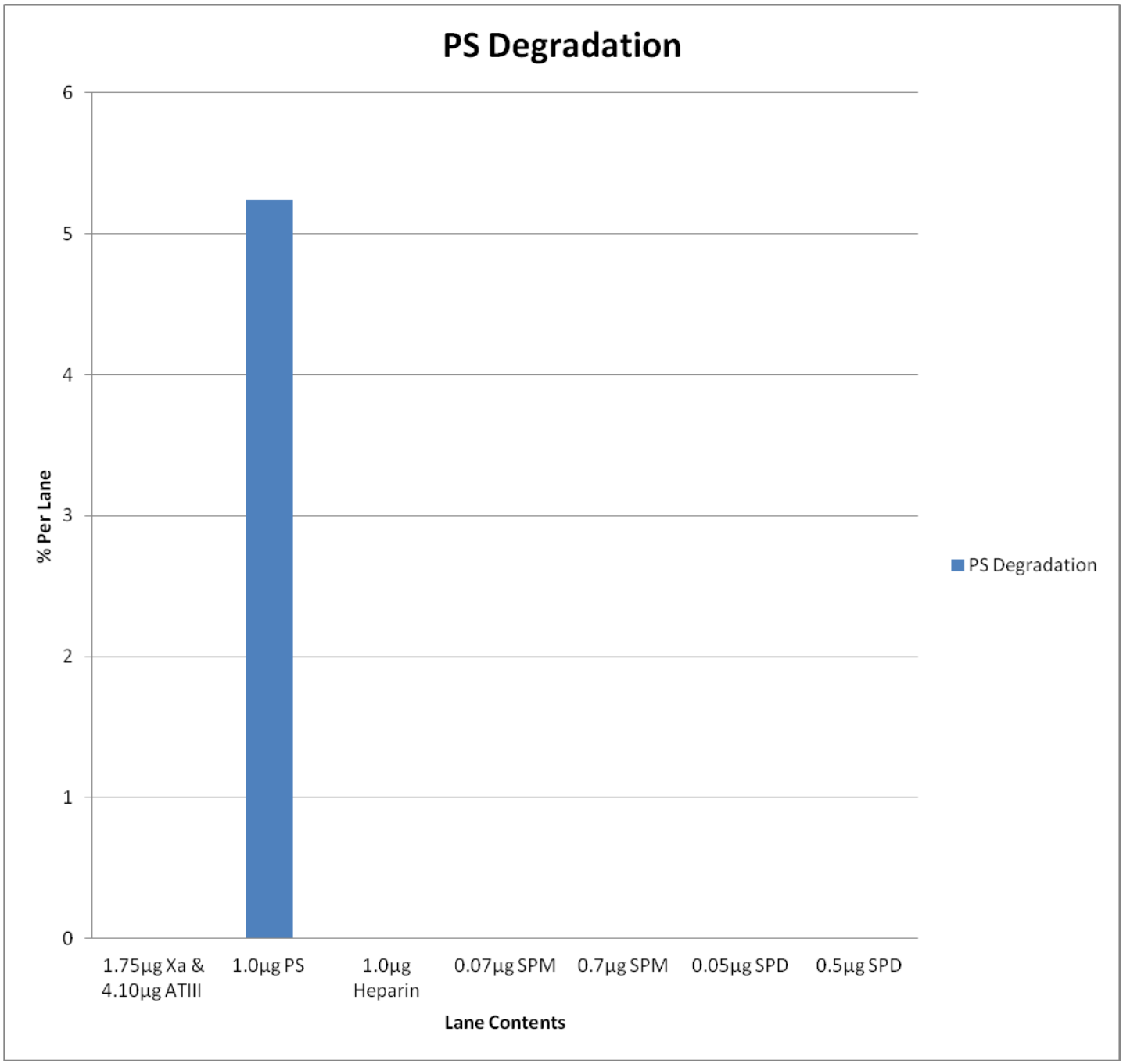


Table 21: PS Degradation Analysis Per Lane
*ND- Not Detected

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
PS Degradation	*ND	5.24	*ND	*ND	*ND	*ND	*ND

Figure W: Graphical Representation of Table 21



Gels 3 and 4 have a slightly different lane set up than Gels 1 and 2

1. 5µL Molecular Weight Markers+ 13µL Tris/NaCl pH8 + 5µL 5X Sample Buffer
2. 2.0µL (1.75µg) FXa + 13µL Tris/NaCl pH8 + 5µL 5X Sample Buffer
3. 2.0µL (4.10µg) ATIII + 13µL Tris/NaCl pH8 + 5µL 5X Sample Buffer
4. 2.0µL (1.75µg) FXa + 2.0µL (4.10µg) ATIII + 11µL Tris/NaCl pH8 + 5µL 5X Sample Buffer
5. [(2.0µL (4.10µg) ATIII + 1µg (3µL) Heparin) 15' at RT, + 1.0µL (1.75µg) FXa + 8µL Tris/NaCl pH8] 60' at RT, + 5µL 5X Sample Buffer]
6. [(2.0µL (4.10µg) ATIII + 1µg (3µL) Protamine Sulfate) 15' at RT, + 2.0µL (1.75µg) FXa + 8µL Tris/NaCl pH8] 60' at RT, + 5µL 5X Sample Buffer]
7. [(2.0µL (4.10µg) ATIII + 0.07µg (3µL) Spermine) 15' at RT, + 2.0µL (1.75µg) FXa + 8µL Tris/NaCl pH8] 60' at RT, + 5µL 5X Sample Buffer]
8. [(2.0µL (4.10µg) ATIII + 0.7µg (3µL) Spermine) 15' at RT, + 2.0µL (1.75µg) FXa + 8µL Tris/NaCl pH8] 60' at RT, + 5µL 5X Sample Buffer]
9. [(2.0µL (4.10µg) ATIII + 0.05µg (3µL) Spermidine) 15' at RT, + 2.0µL (1.75µg) FXa + 8µL Tris/NaCl pH8] 60' at RT, + 5µL 5X Sample Buffer]
10. [(2.0µL (4.10µg) ATIII + 0.5µg (3µL) Spermidine) 15' at RT, + 2.0µL (1.75µg) FXa + 8µL Tris/NaCl pH8] 60' at RT, + 5µL 5X Sample Buffer]

Figure X: Gel 3

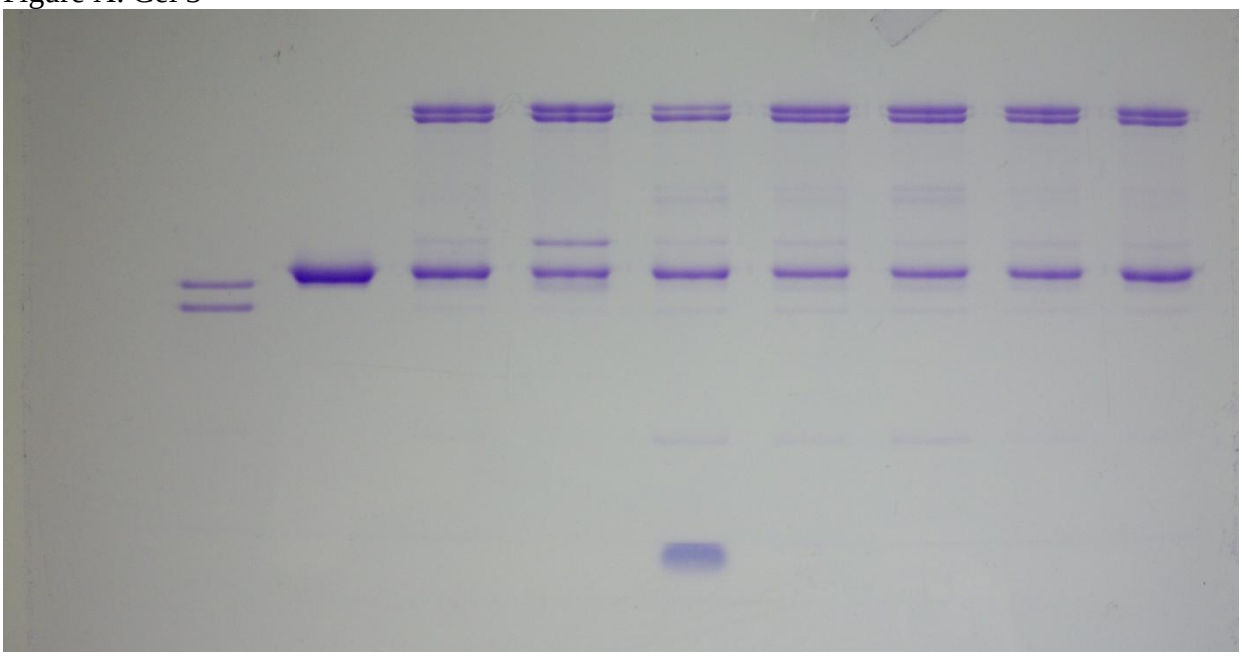
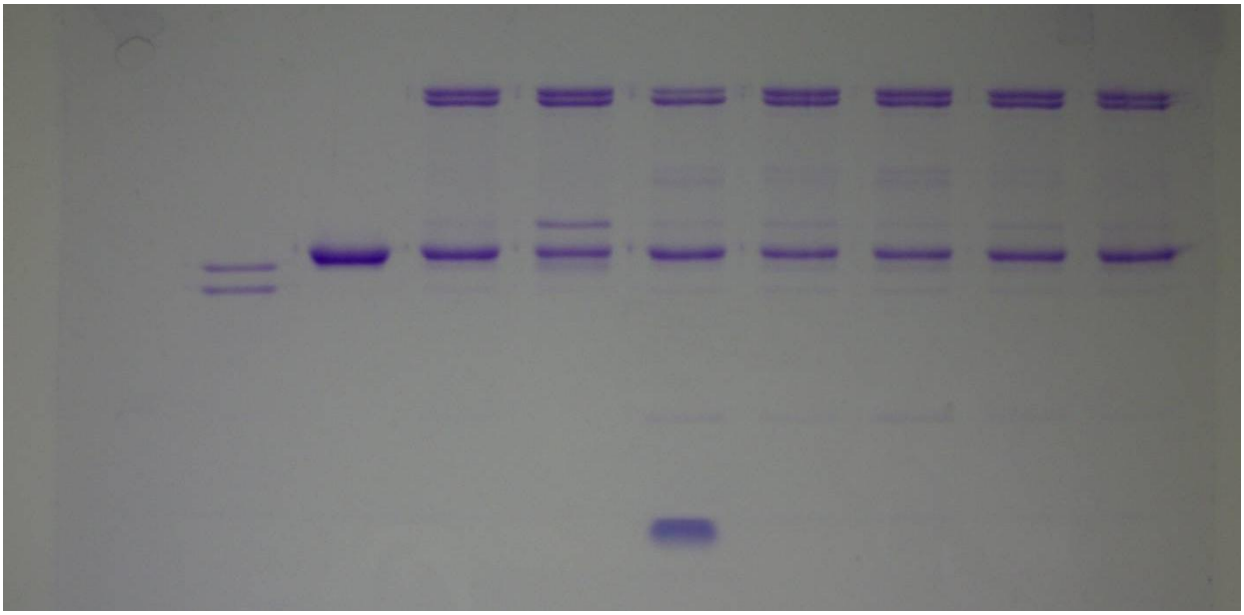


Figure Y: Gel 4



Gel 3:

Table 22: Overall Protein Per Lane

*ND= Not Detected

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Primary Alpha	30.4	30.5	14.6	32.5	33.2	31.3	29.5
Primary Beta	24.6	26.6	25.3	27.5	26.5	27.4	25
Secondary Alpha	*ND	*ND	*ND	*ND	*ND	*ND	*ND
Seconday Beta	*ND	*ND	*ND	*ND	*ND	*ND	*ND
Tertiary Alpha	0.458	0.508	0.644	0.474	1.19	0.527	0.147
Tertiary Beta	0.619	0.219	1.25	0.372	1.32	0.422	0.071
ATIIIM	1.84	11.5	1.6	2.29	0.928	1.28	0.938
ATIII	40.5	28.4	36	33.8	33.2	37.5	42.8
Xa Alpha	0.298	1.34	0.885	0.852	0.746	0.397	1.11
Xa Beta	0.962	0.328	0.111	1.43	1.13	0.66	0.27
Solvent Front	0.375	0.586	1.34	0.83	1.71	0.545	0.141
PS Degradation	*ND	*ND	18.3	*ND	*ND	*ND	*ND

Standard Deviation	14.32971916	Mean	10.93817188	Standard Error	1.65465344

Figure Z: Graphical Representation of Table 22

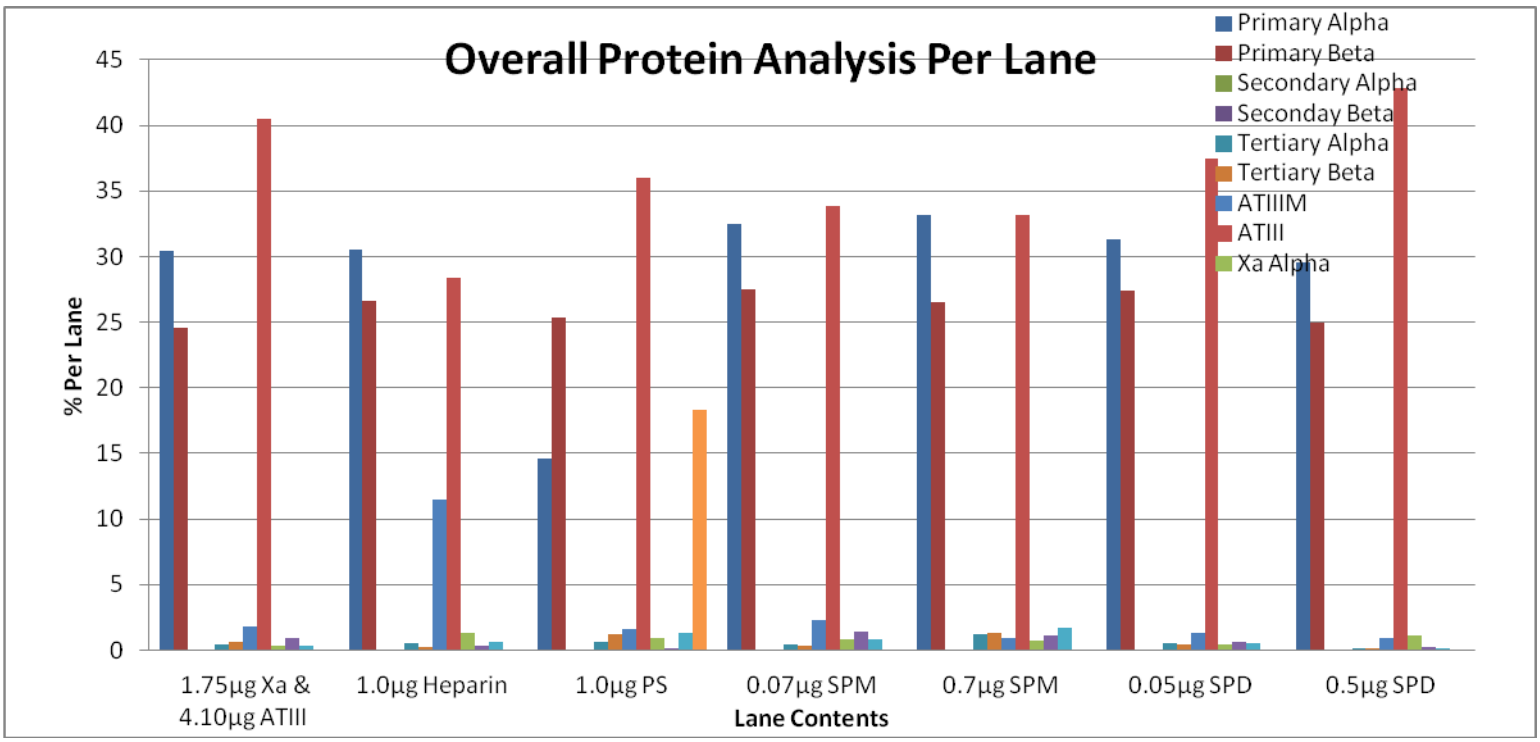


Table 23: Primary Alpha [Xa-ATIII] Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Primary Alpha	30.4	30.5	14.6	32.5	33.2	31.3	29.5

Figure AA: Graphical Representation of Table 23

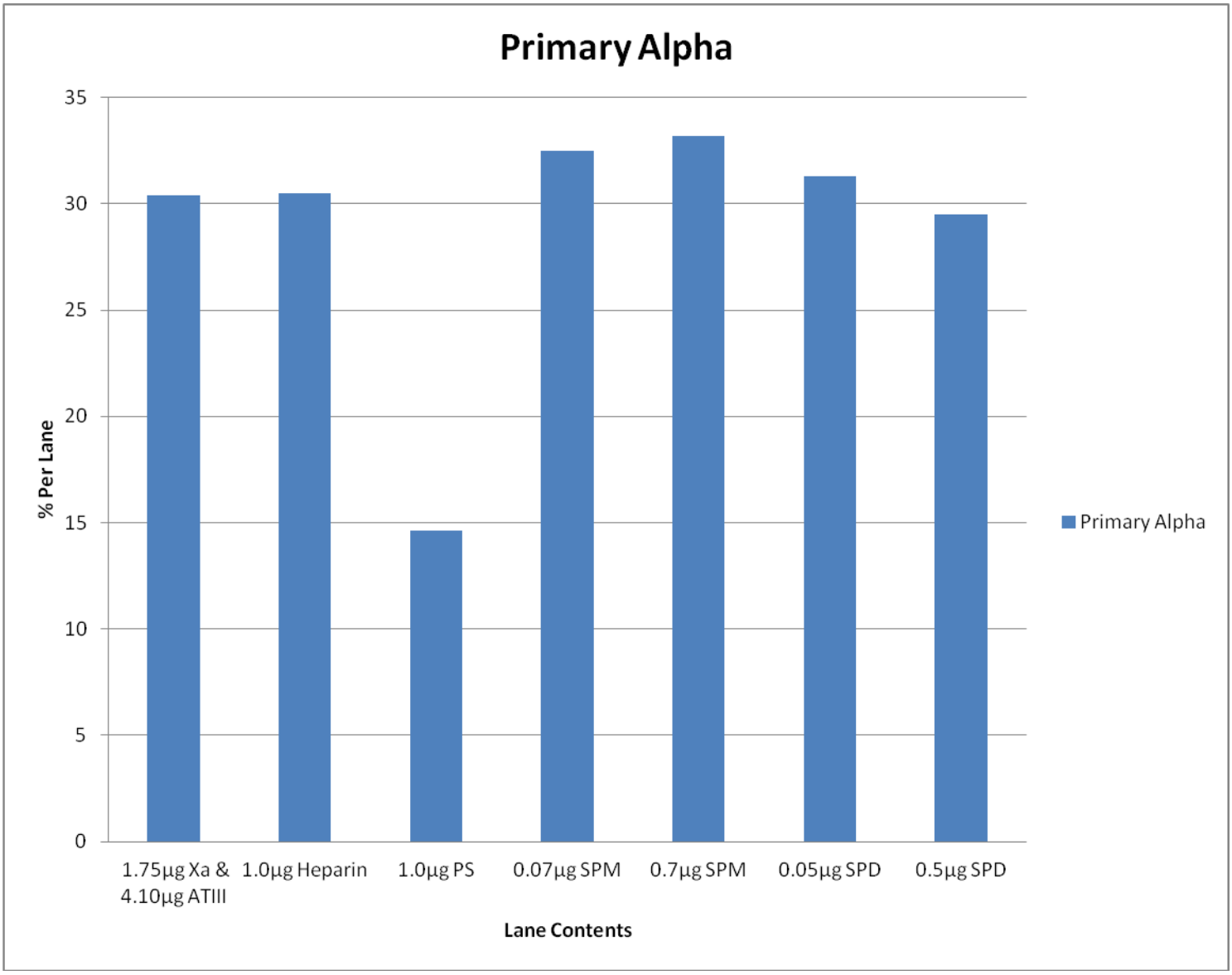


Table 24: Primary Beta [Xa-ATIII] Complex Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Primary Beta	24.6	26.6	25.3	27.5	26.5	27.4	25

Figure BB: Graphical Representation of Table 24

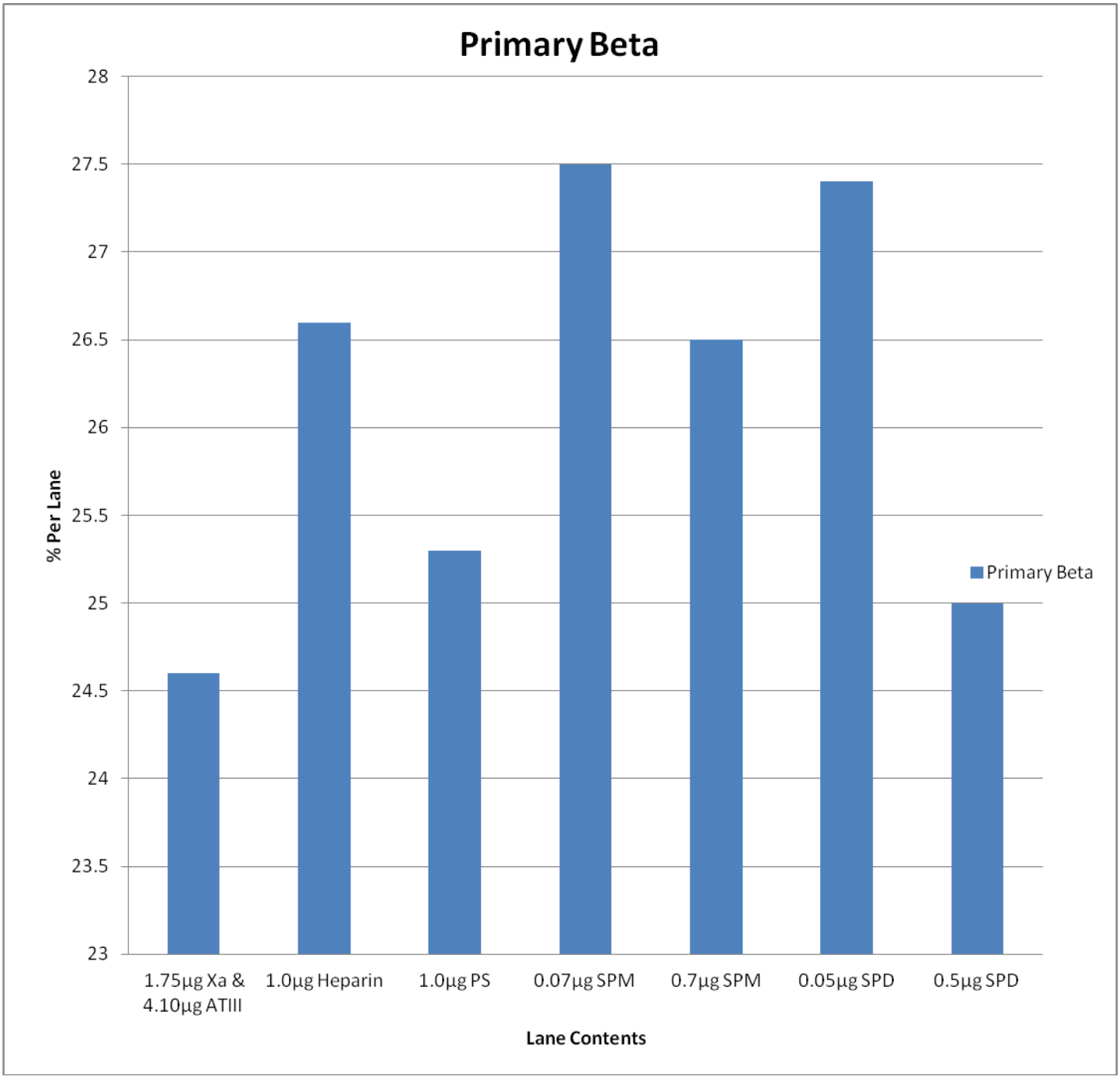


Table 25: Tertiary Alpha [Xa-ATIII] Complex Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Tertiary Alpha	0.458	0.508	0.644	0.474	1.19	0.527	0.147

Figure CC: Graphical Representation of Table 25

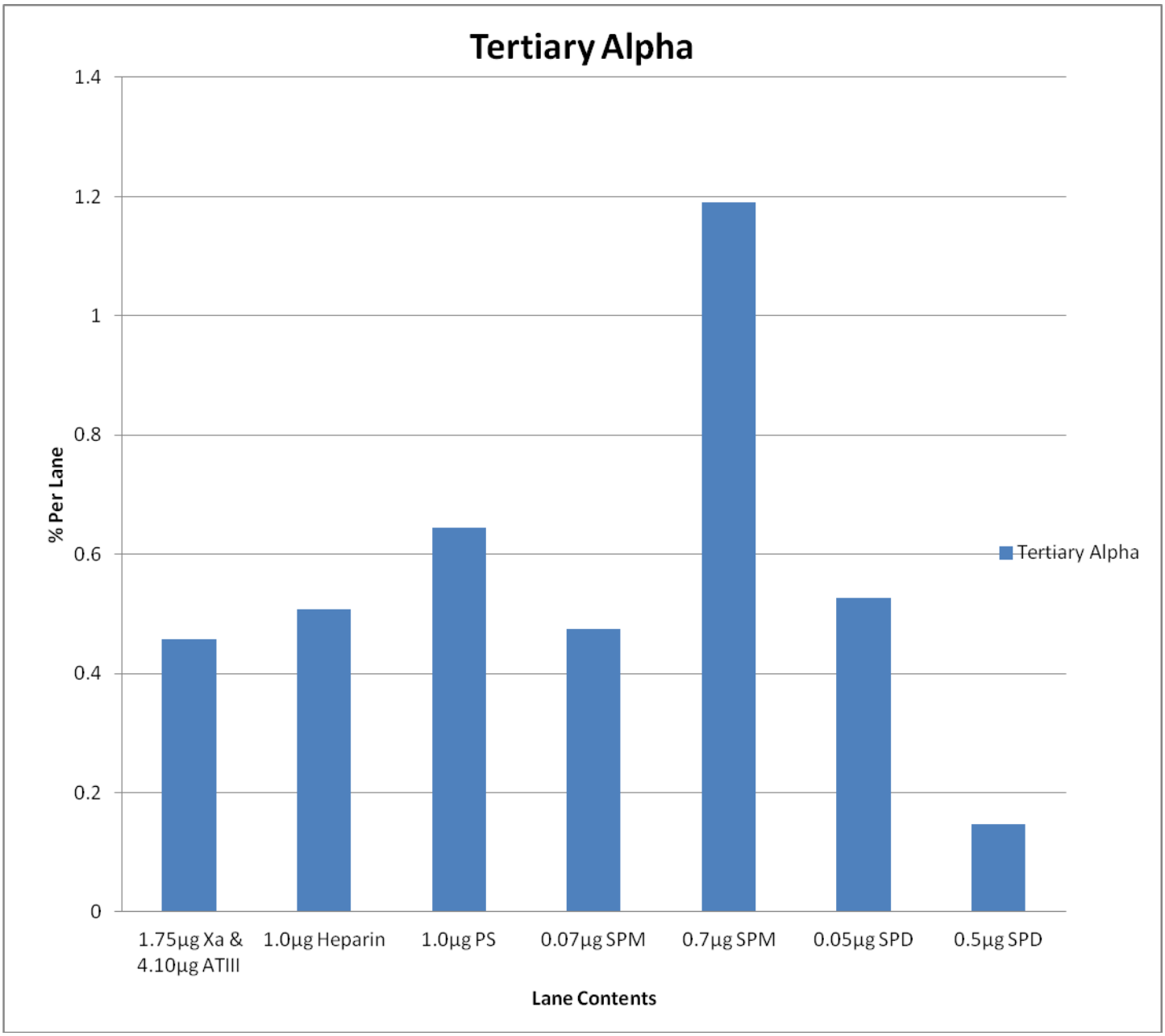


Table 26: Tertiary Beta [IIa-ATIII] Complex Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Tertiary Beta	0.619	0.219	1.25	0.372	1.32	0.422	0.071

Figure DD: Graphical Representation of Table 26

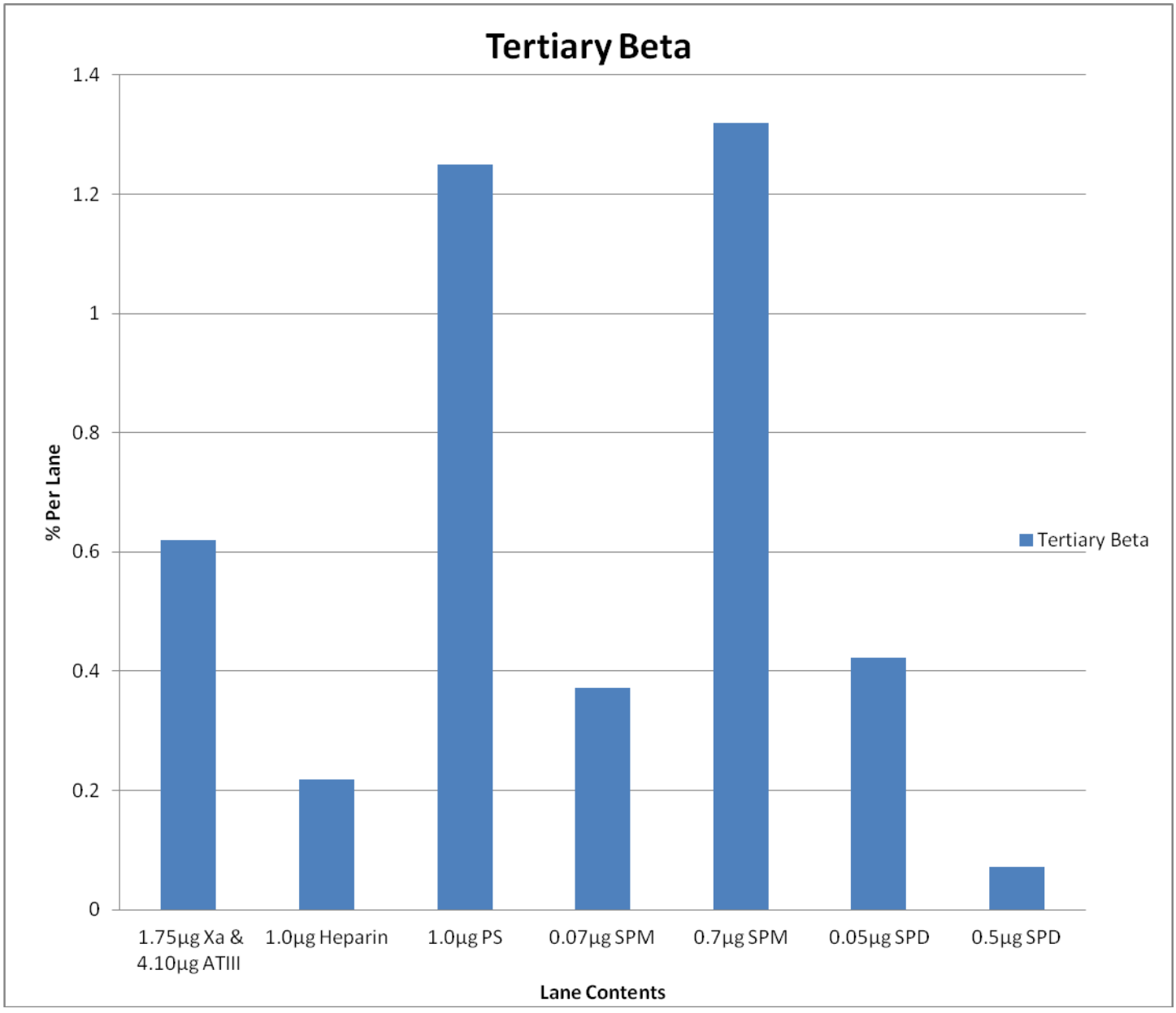


Table 27: ATIII-M Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIIIM	1.84	11.5	1.6	2.29	0.928	1.28	0.938

Figure EE: Graphical Representation of Table 27

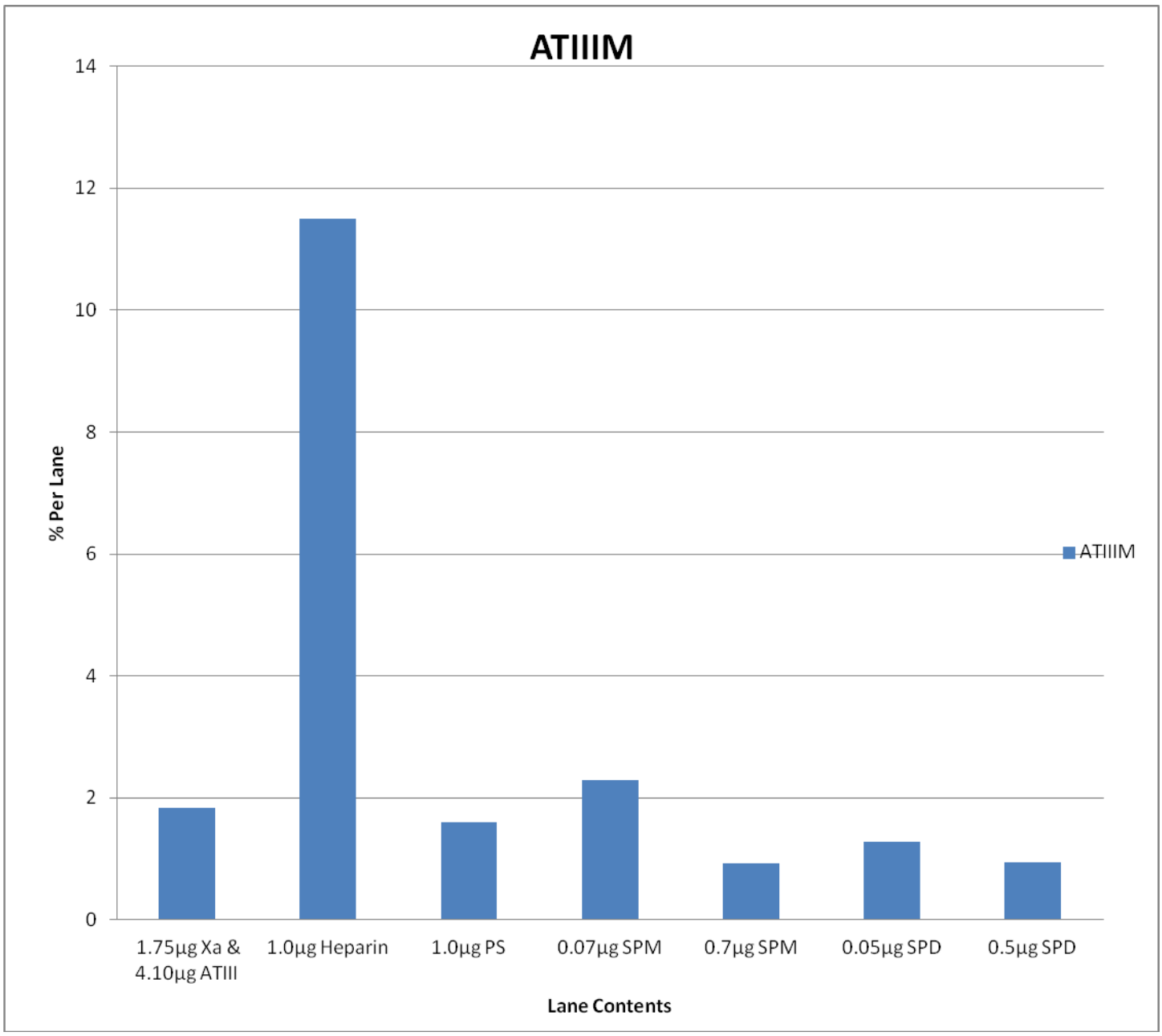


Table 28: ATIII Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIII	40.5	28.4	36	33.8	33.2	37.5	42.8

Figure FF: Graphical Representation of Table 28

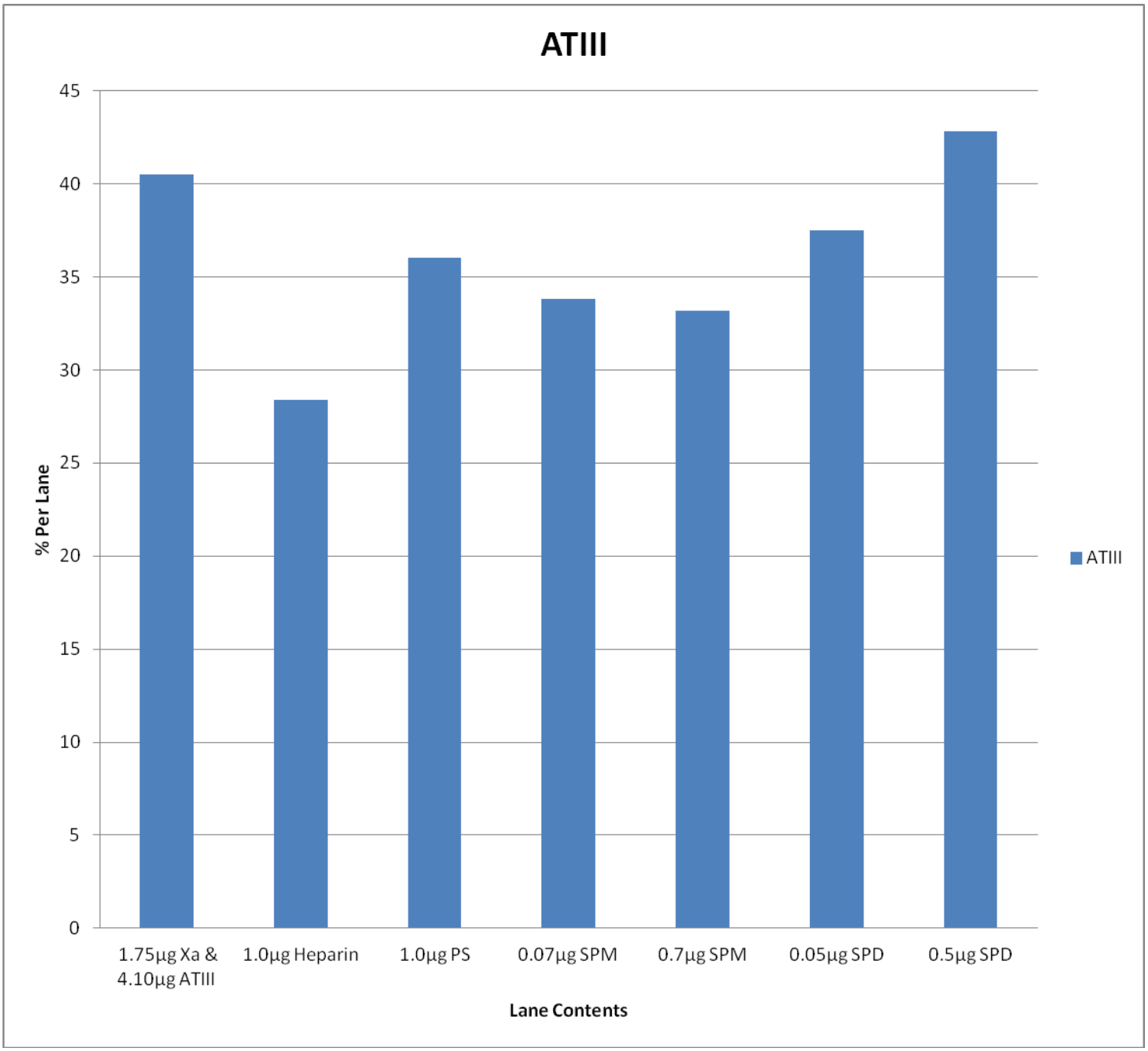


Table 29: Xa Alpha Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Xa Alpha	0.298	1.34	0.885	0.852	0.746	0.397	1.11

Figure GG: Graphical Representation of Table 29

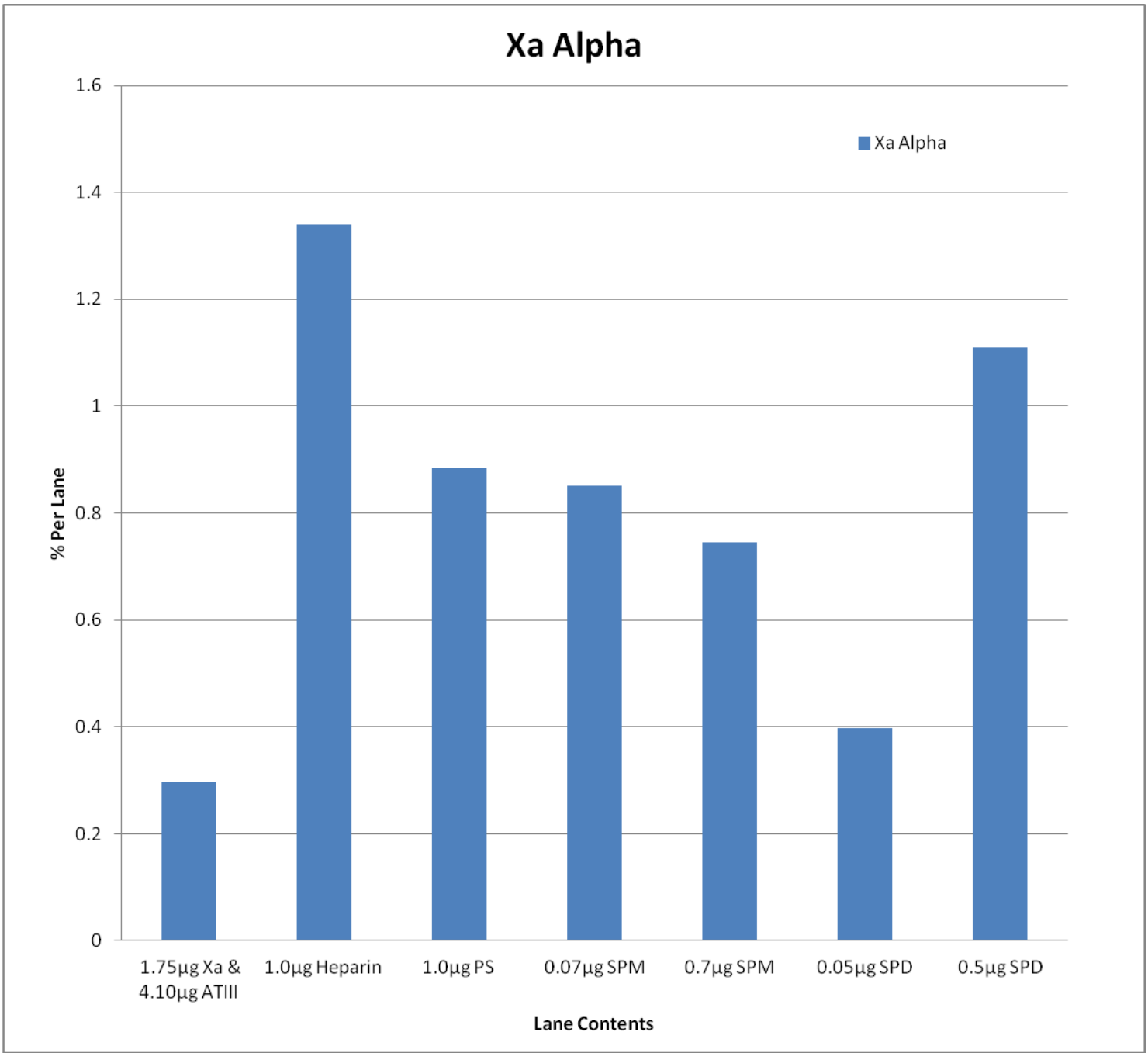


Table 30: Xa Beta Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Xa Beta	0.962	0.328	0.111	1.43	1.13	0.66	0.27

Figure HH: Graphical Representation of Table 30

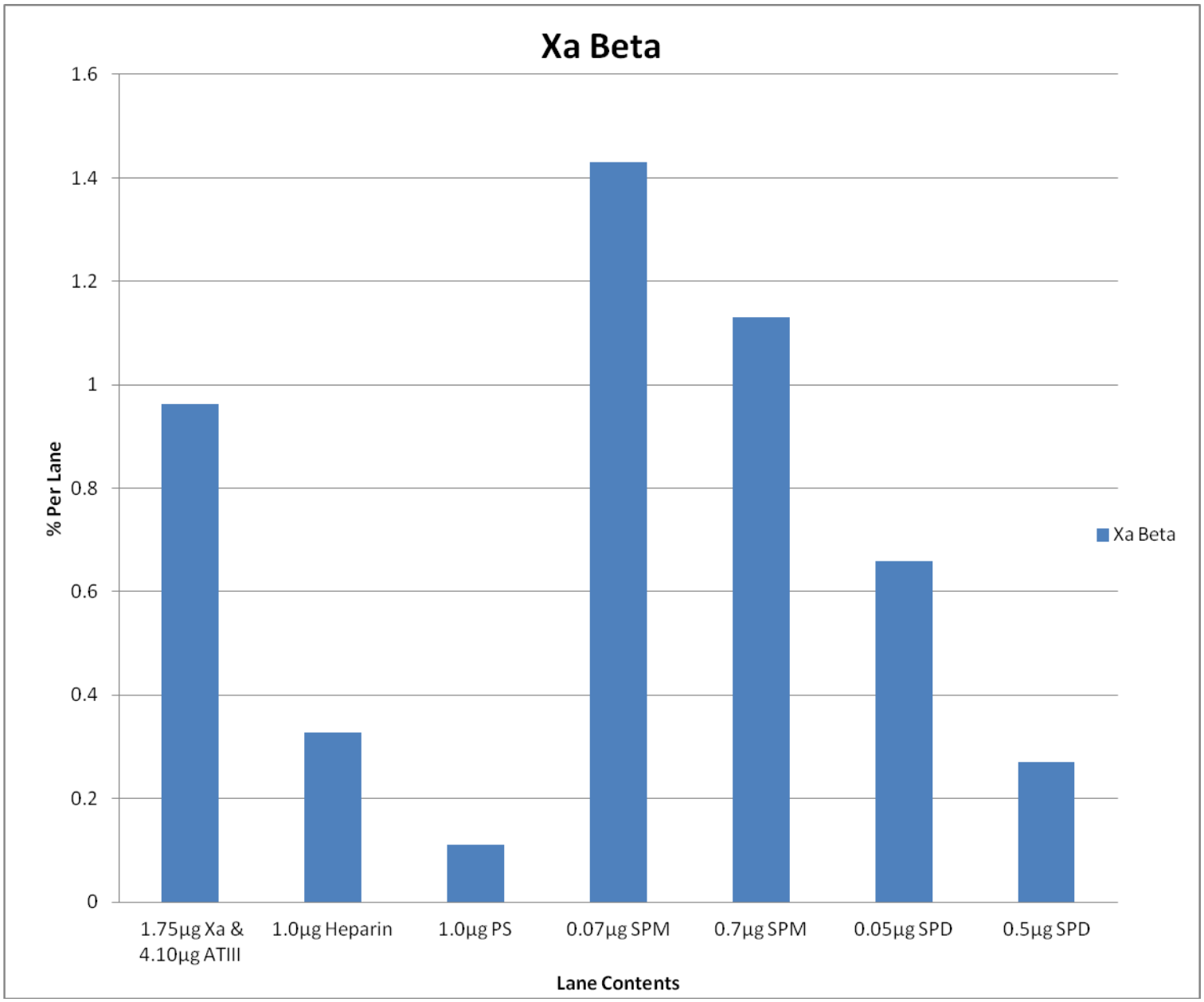


Table 31: Solvent Front Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Solvent Front	0.375	0.586	1.34	0.83	1.71	0.545	0.141

Figure II: Graphical Representation of Table 31

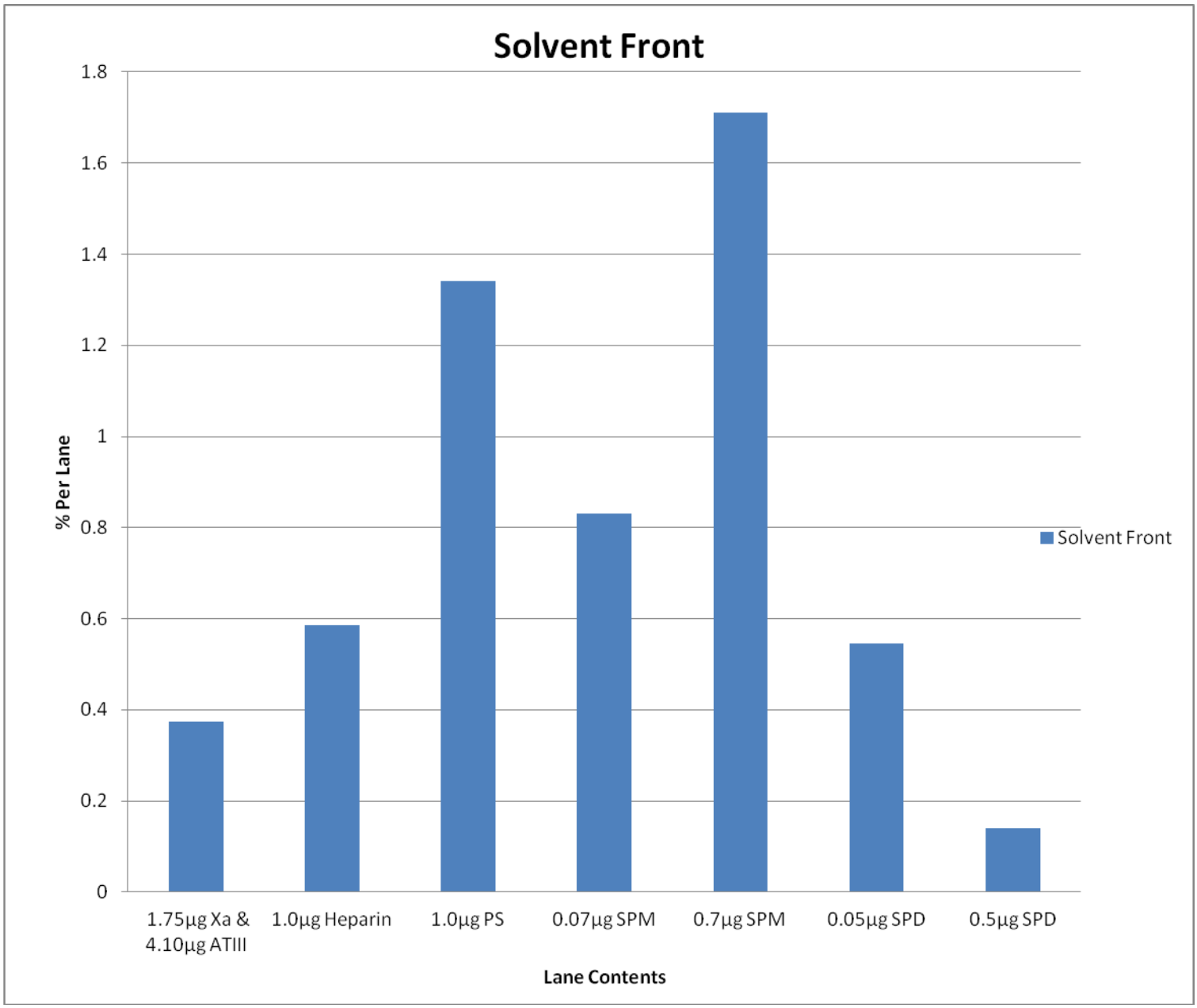
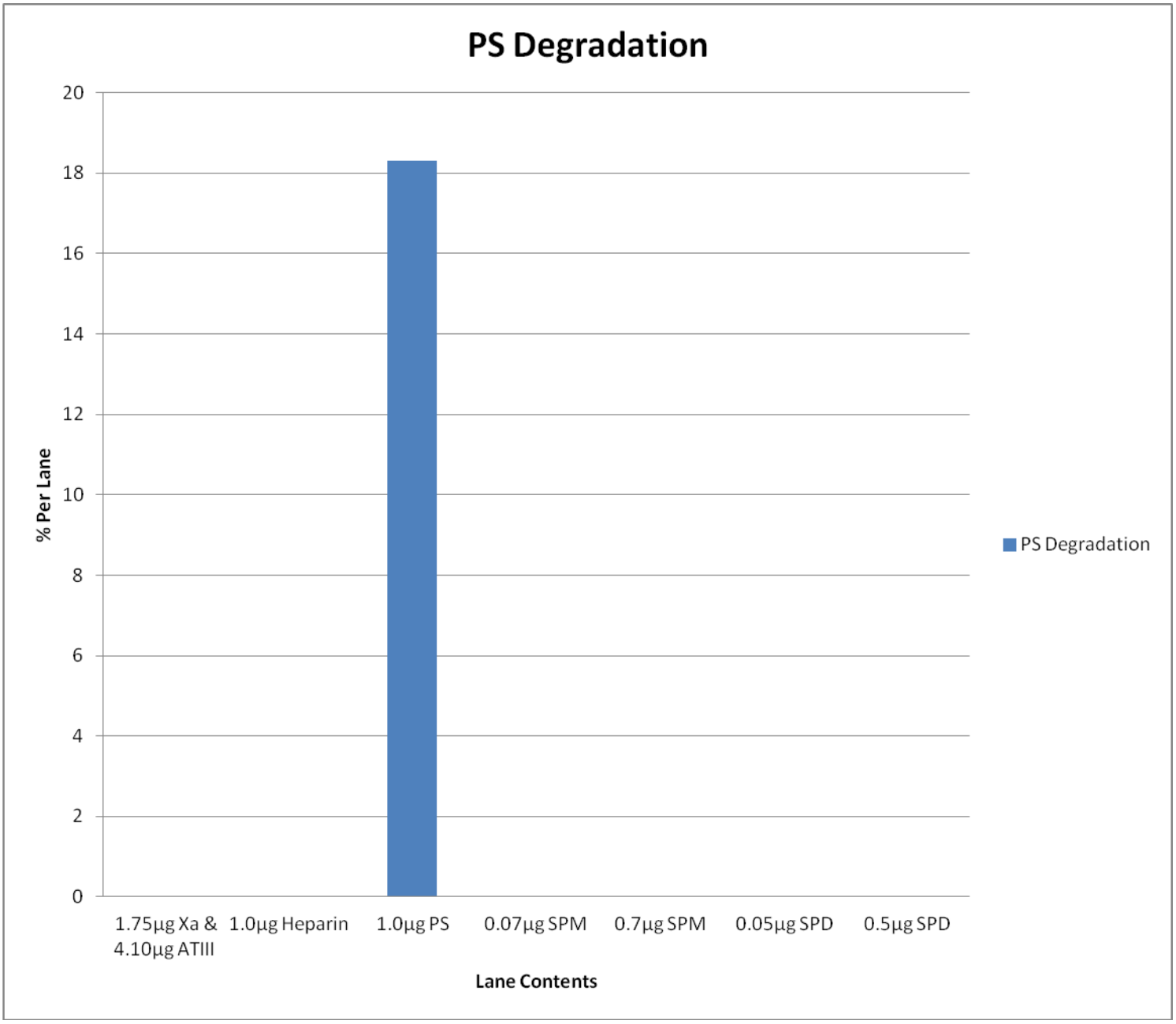


Table 32: PS Degradation Analysis Per Lane
*ND= Not Detected

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
PS Degradation	*ND	*ND	18.3	*ND	*ND	*ND	*ND

Figure JJ: Graphical Representation of Table 32



Gel 4

Table 33: Overall Protein Analysis Per Lane

*ND= Not Detected

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Primary Alpha	28.4	30.8	11	30.2	29	30.9	25.7
Primary Beta	23.7	26.6	23.5	27	24.1	25.5	23.6
Secondary Alpha	*ND	*ND	*ND	*ND	*ND	*ND	*ND
Seconday Beta	*ND	*ND	*ND	*ND	*ND	*ND	*ND
Tertiary Alpha	0.15	0.141	0.269	0.783	2.72	0.425	0.241
Tertiary Beta	0.229	0.114	0.872	0.89	1.78	0.498	0.18
ATIIIM	0.768	12	0.907	2.564	3.08	1.17	1.24
ATIII	44.4	28.7	34.2	34.4	33	40.4	47.5
Xa Alpha	0.84	0.8	0.253	1.01	1	0.227	0.647
Xa Beta	0.685	0.72	0.618	1.253	3.29	0.532	0.486
Solvent Front	0.773	0.125	1.29	1.915	2.04	0.363	0.433
PS Degradation	*ND	*ND	27.2	*ND	*ND	*ND	*ND

Standard Deviation	14.36258482	Mean	10.93939063	Standard Error	1.65844844

Figure KK: Graphical Representation of Table 33

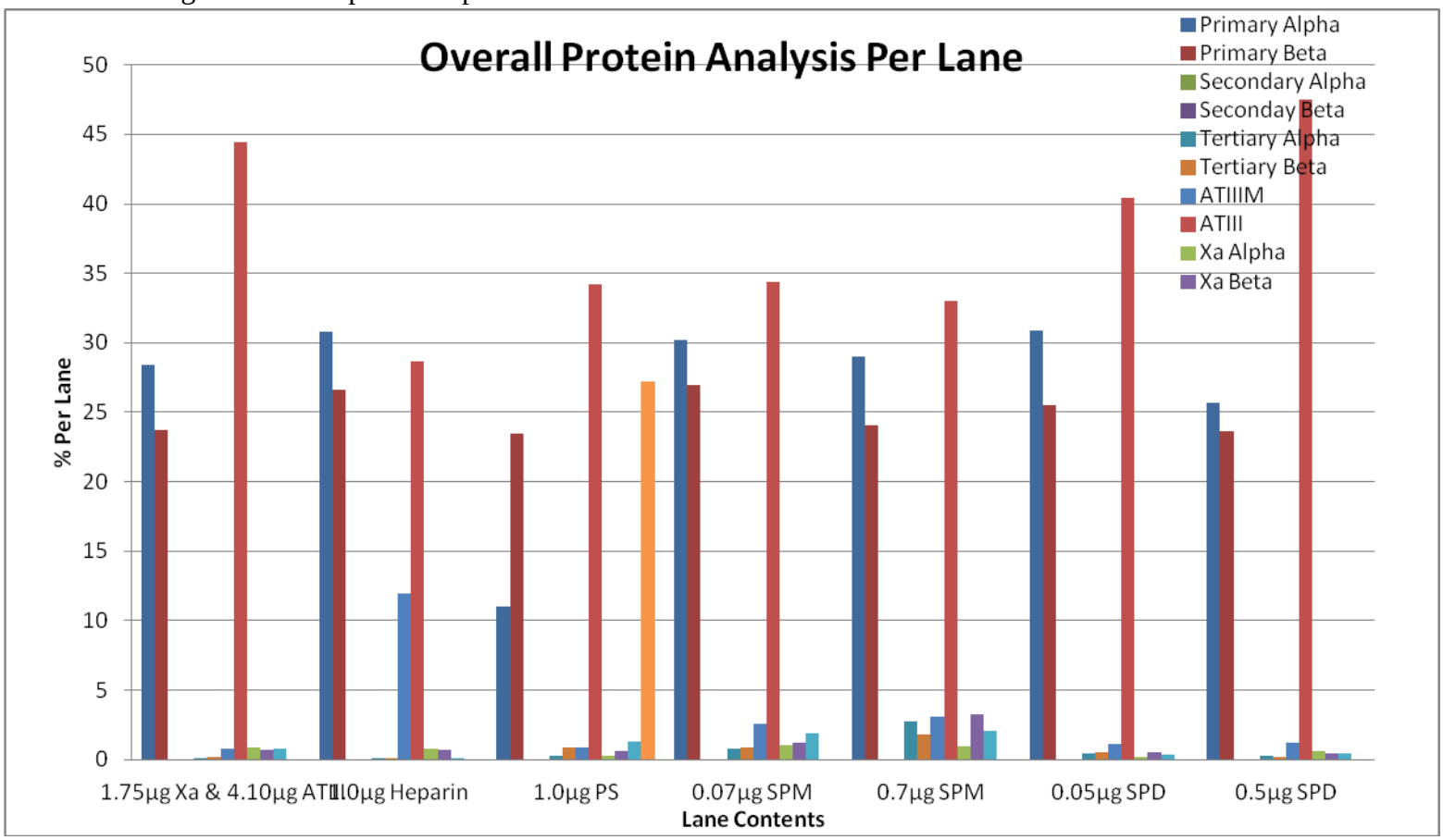


Table 34: Primary Alpha [Xa-ATIII] Complex Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Primary Alpha	28.4	30.8	11	30.2	29	30.9	25.7

Figure LL: Graphical Representation of Table 34

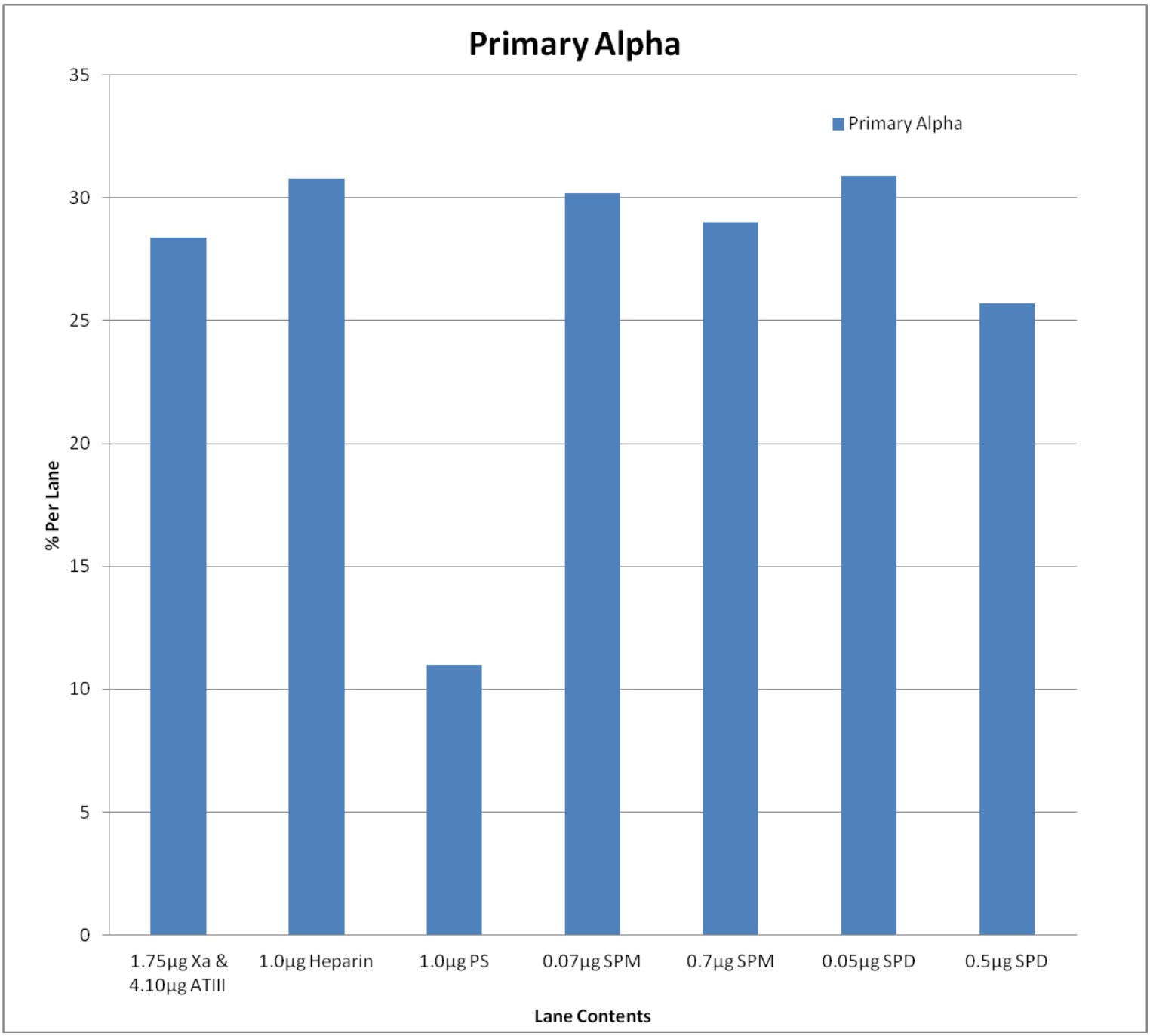


Table 35: Primary Beta [Xa-ATIII] Complex Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Primary Beta	23.7	26.6	23.5	27	24.1	25.5	23.6

Figure MM: Graphical Representation of Table 35

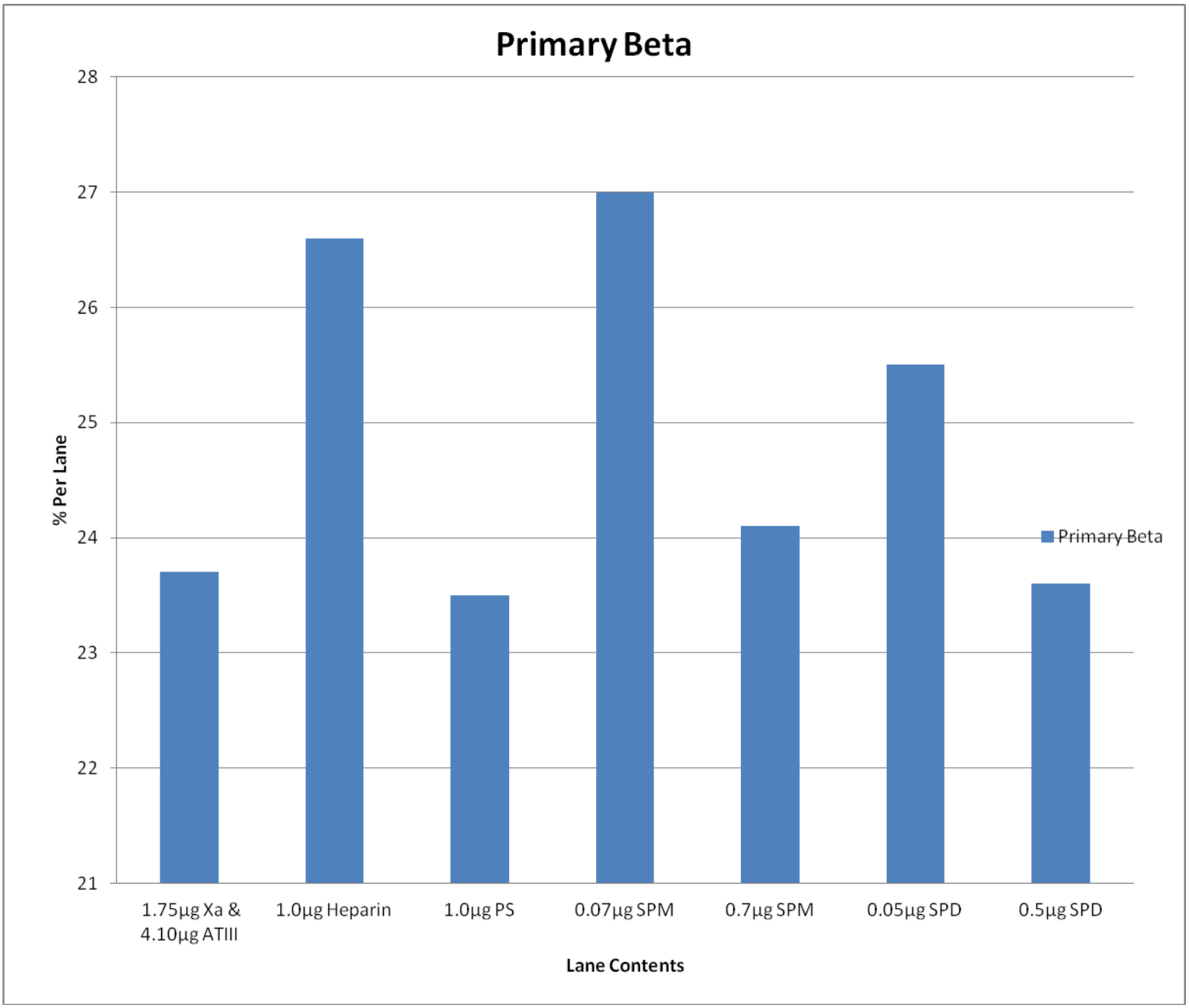


Table 36: Tertiary Alpha [IIa-ATIII] Complex Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Tertiary Alpha	0.15	0.141	0.269	0.783	2.72	0.425	0.241

Figure NN: Graphical Representation of Table 36

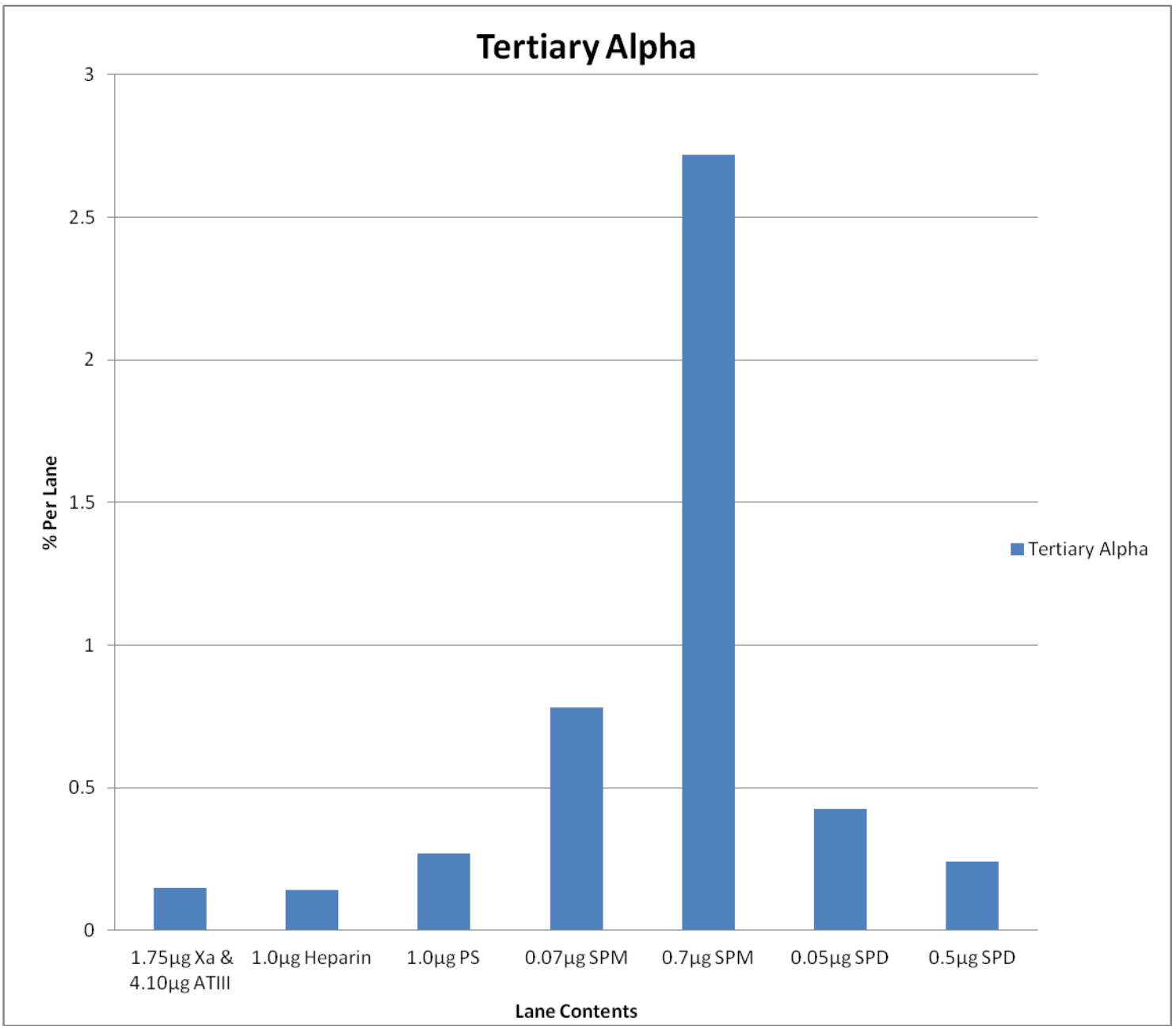


Table 37: Tertiary Beta [Xa-ATIII] Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Tertiary Beta	0.229	0.114	0.872	0.89	1.78	0.498	0.18

Figure OO: Graphical Representation of Table 37

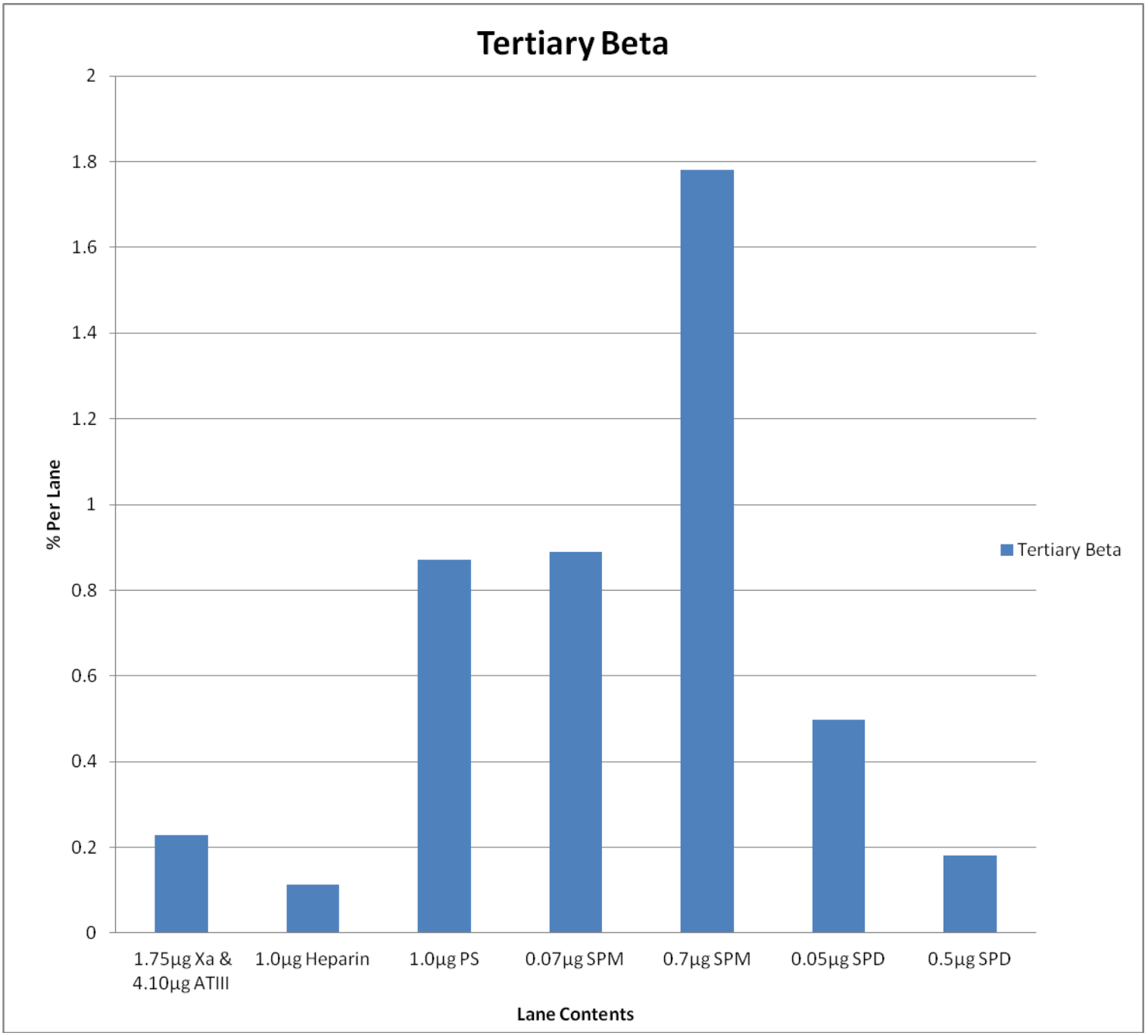


Table 38: ATIII-M Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIIIM	0.768	12	0.907	2.564	3.08	1.17	1.24

Figure PP: Graphical Representation of Table 38

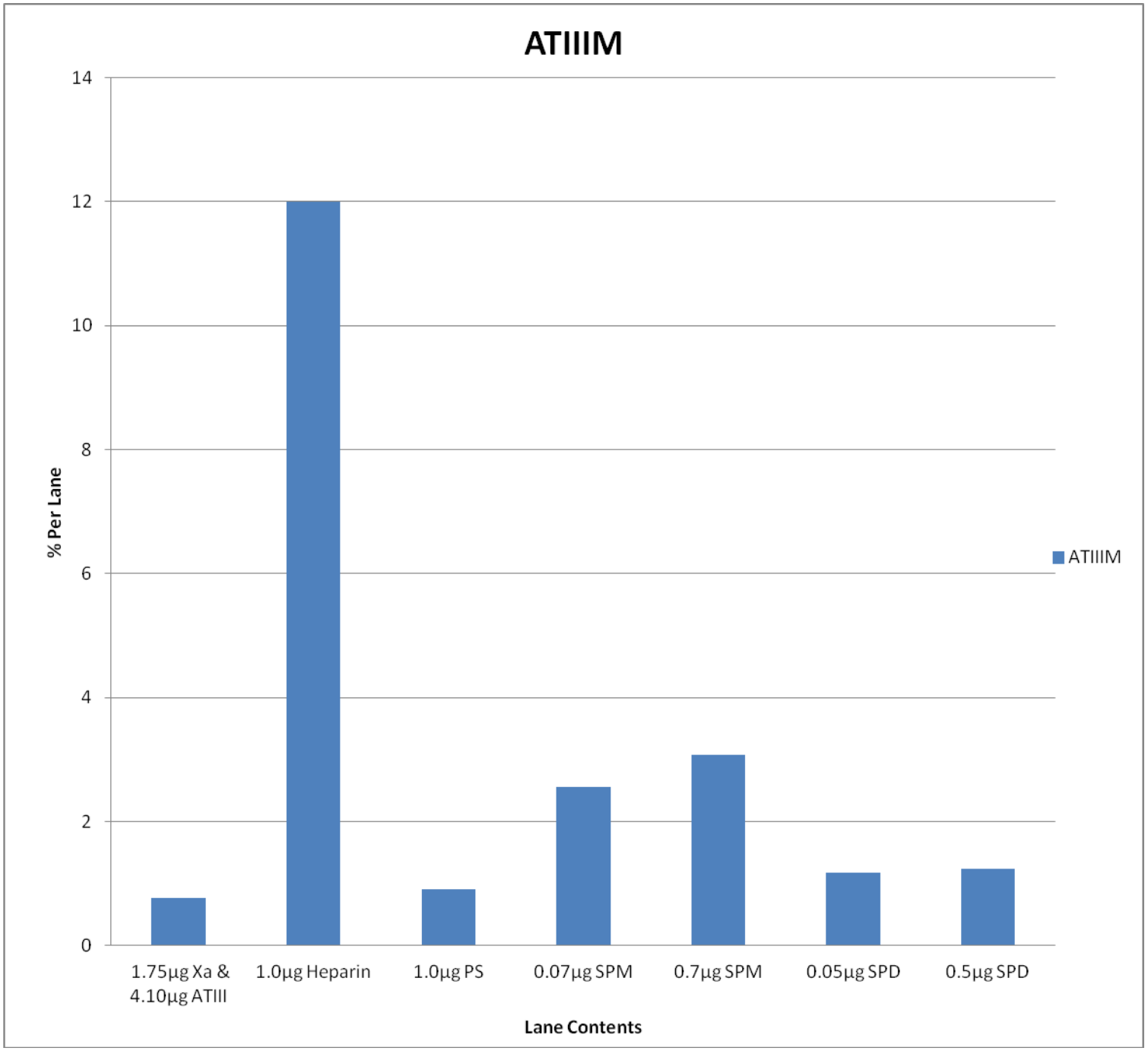


Table 39: ATIII Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIII	44.4	28.7	34.2	34.4	33	40.4	47.5

Figure QQ: Graphical Representation of Table 39

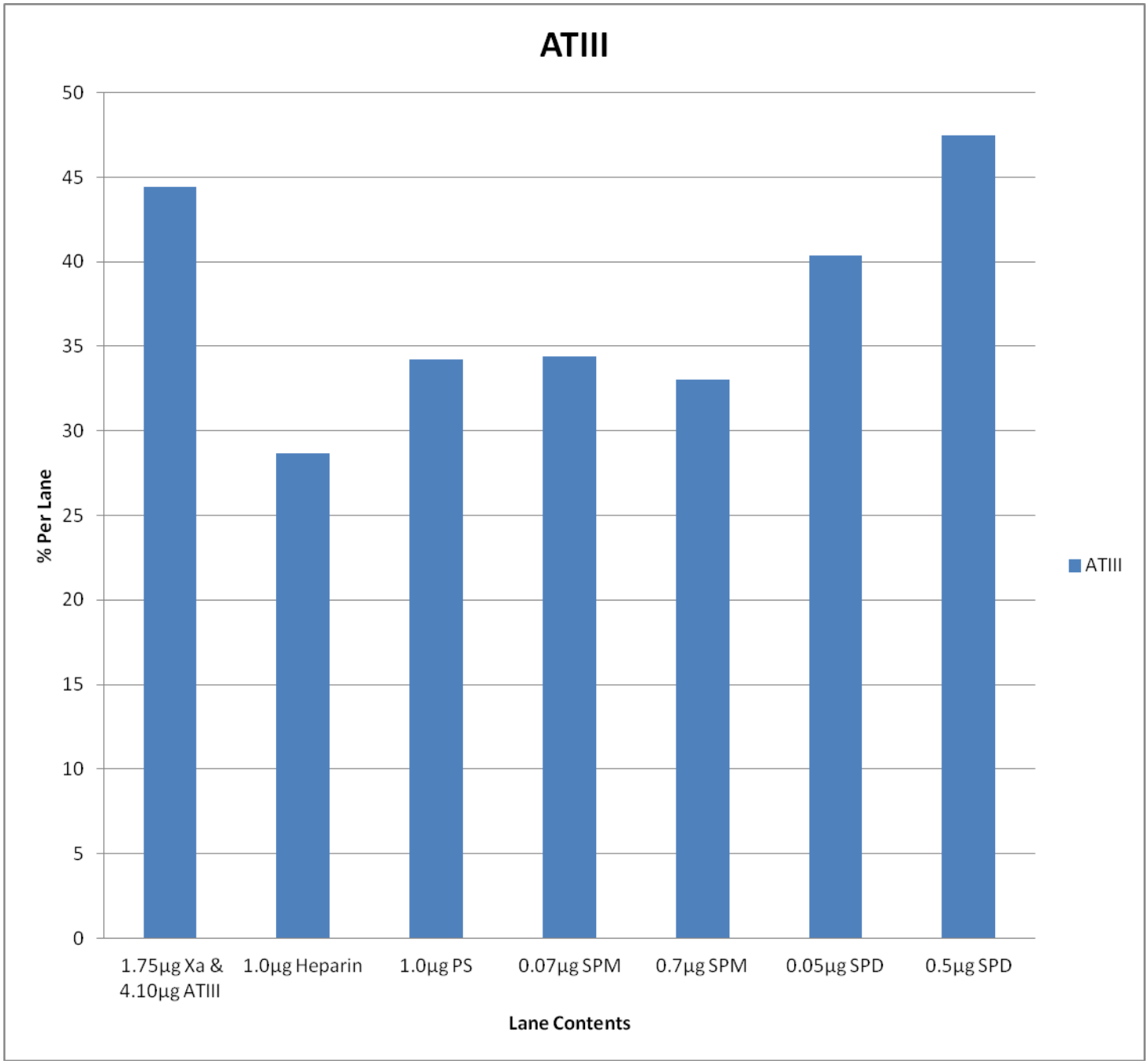


Table 40: Xa Alpha Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Xa Alpha	0.84	0.8	0.253	1.01	1	0.227	0.647

Figure RR: Graphical Representation of Table 40

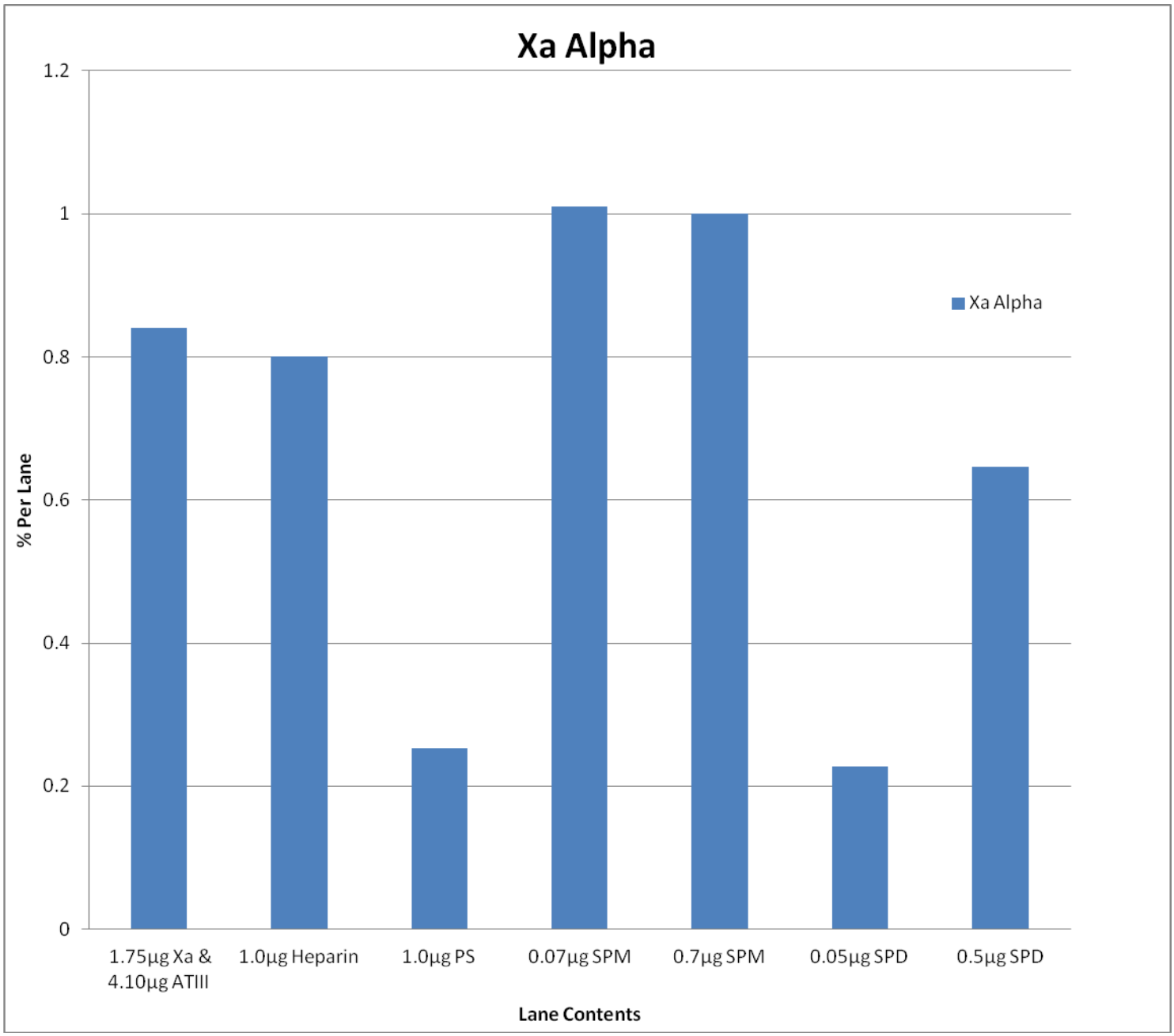


Table 41: Xa Beta Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Xa Beta	0.685	0.72	0.618	1.253	3.29	0.532	0.486

Figure SS: Graphical Representation of Table 41

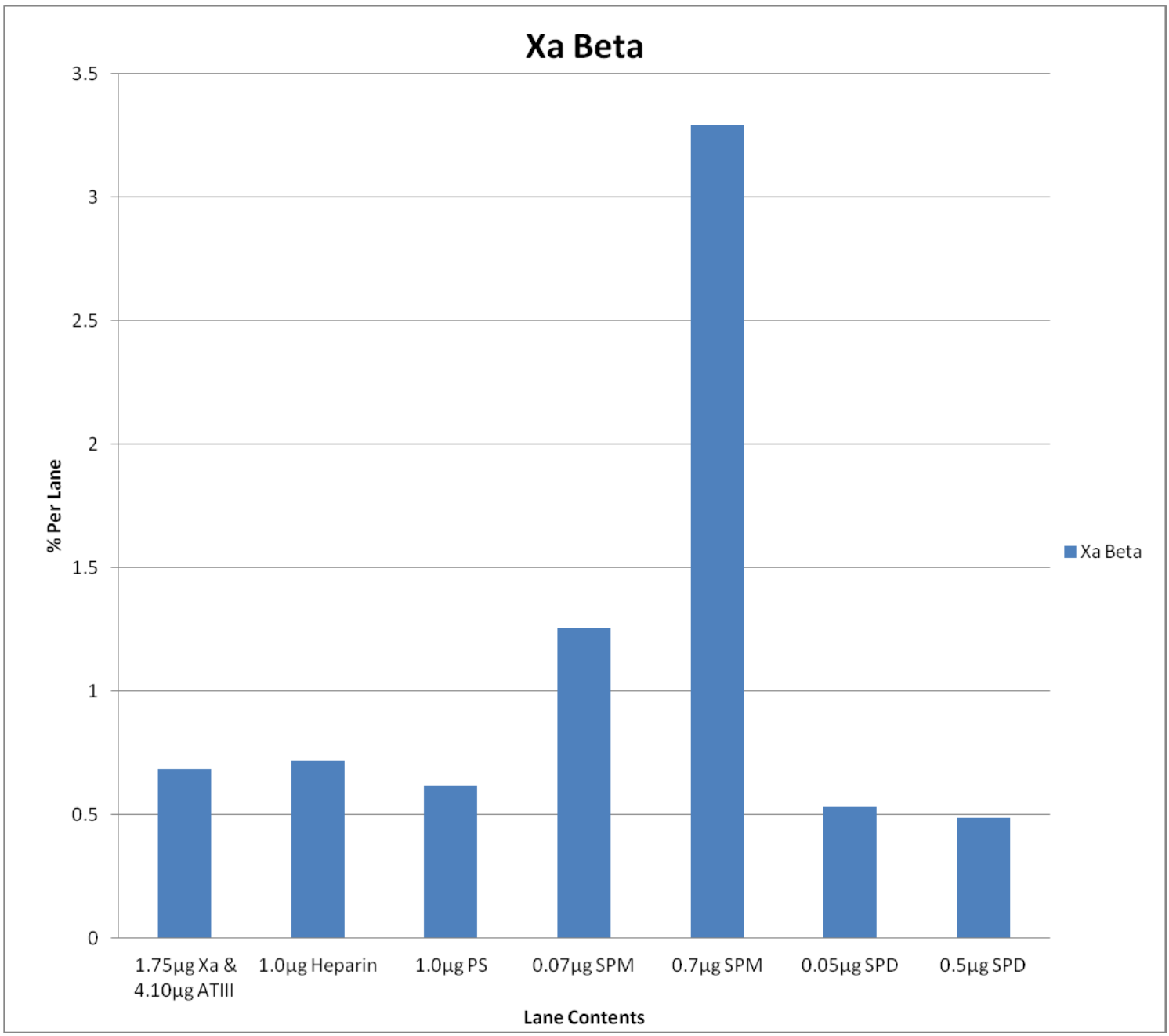


Table 42: Solvent Front Analysis Per Lane

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Solvent Front	0.773	0.125	1.29	1.915	2.04	0.363	0.433

Figure TT: Graphical Representation of Table 42

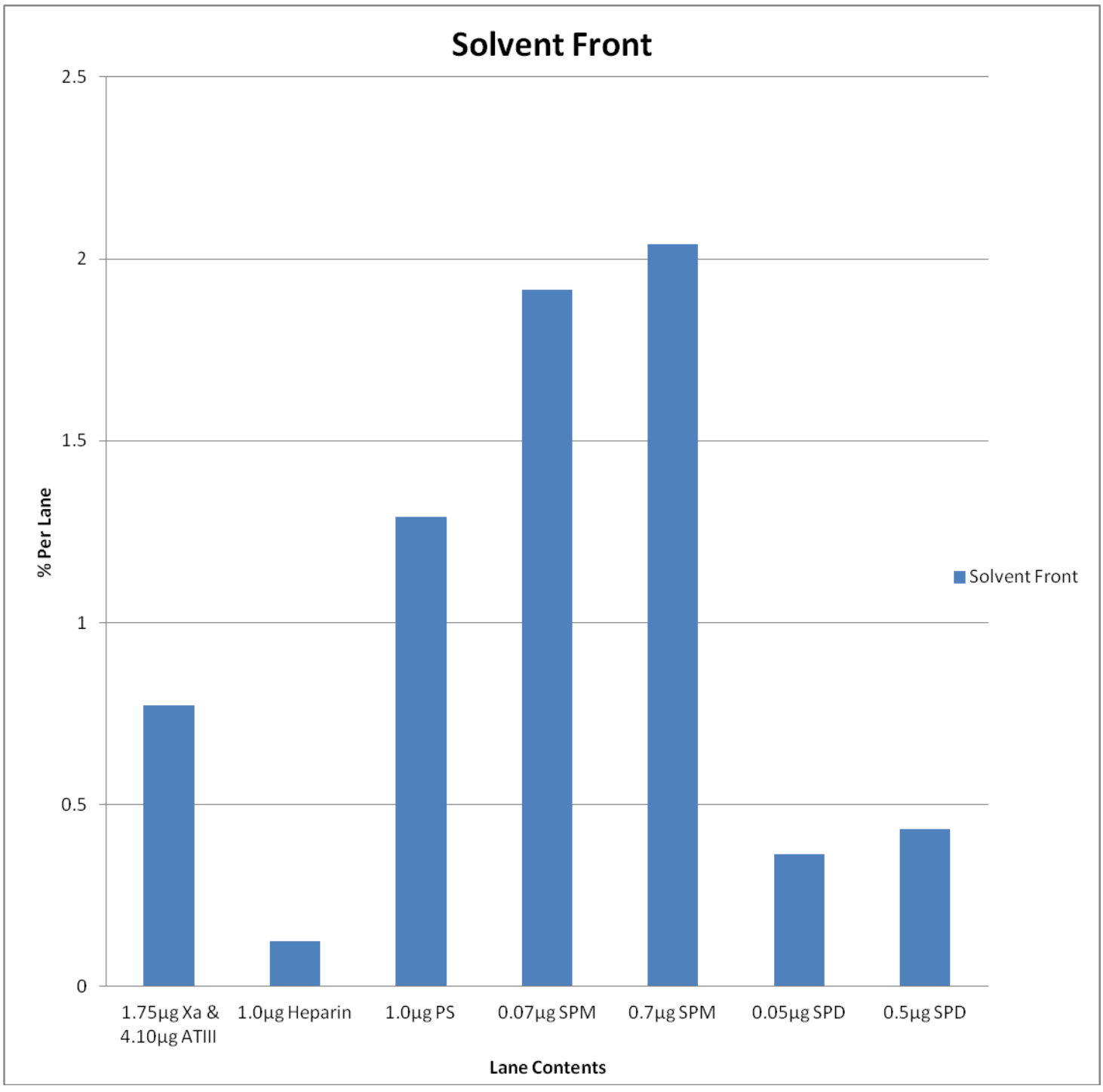
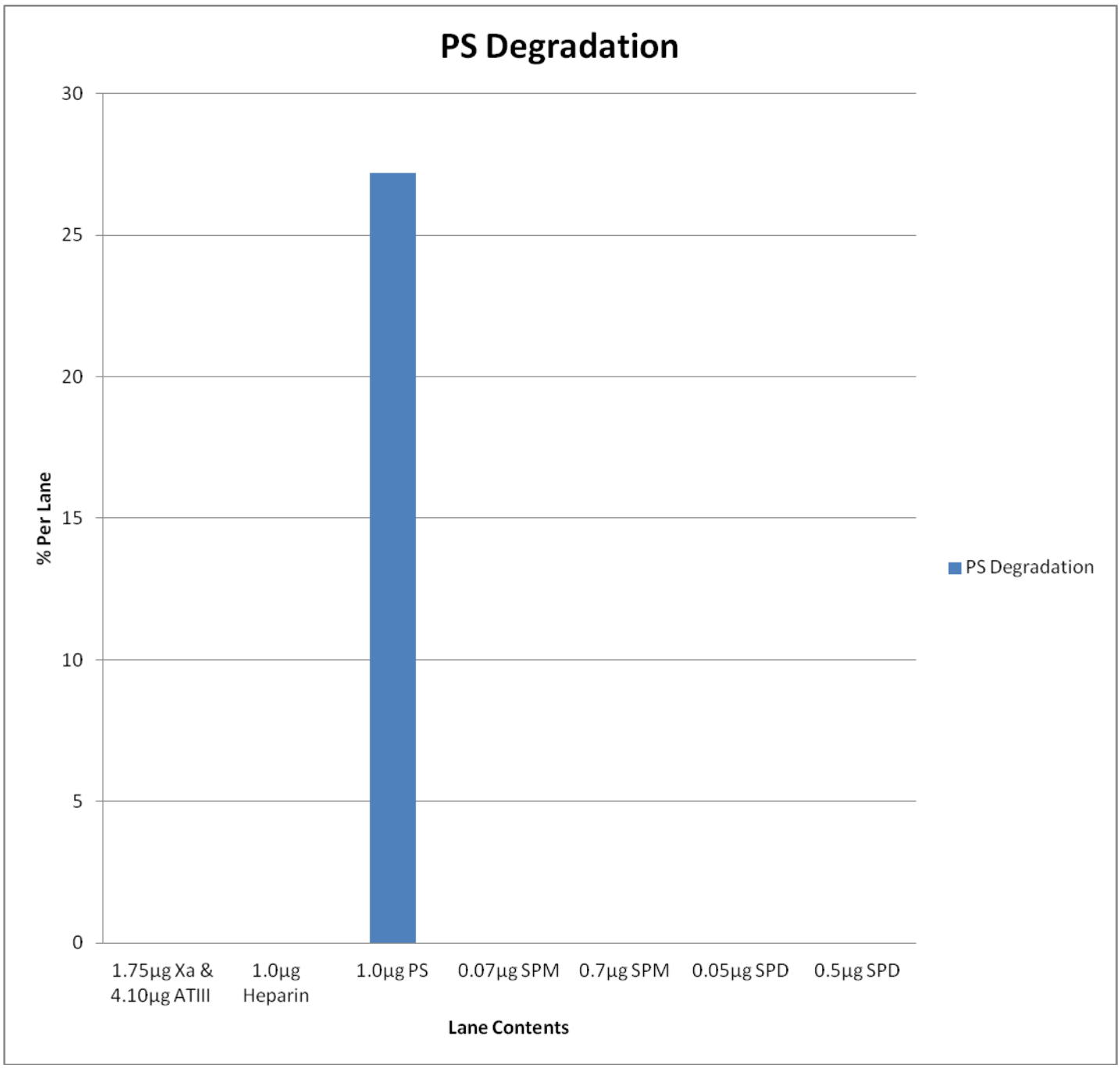


Table 43: PS Degradation Analysis Per Lane
*ND= Not Detected

	1.75µg Xa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
PS Degradation	*ND	*ND	27.2	*ND	*ND	*ND	*ND

Figure UU; Graphical Representation of Table 43



Tables used to determine P Values

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Primary Alpha	37.13	22.9	42.14	39.8	36.2	37.3	28.4
Primary Alpha	36.2	24	39.2	35.8	33.7	37.4	29.4
Primary Alpha	30.4	14.6	30.5	32.5	33.2	31.3	29.5
Primary Alpha	28.4	11	30.8	30.2	29	30.9	25.7

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Primary Beta	28.75	27.4	32.63	29.1	25.9	28.1	21.1
Primary Beta	28.1	26.9	32.7	30.5	28.6	26.9	22
Primary Beta	24.6	25.3	26.6	27.5	26.5	27.4	25
Primary Beta	23.7	23.5	26.6	27	24.1	25.5	23.6

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Secondary Alpha	*ND	*ND	*ND	*ND	*ND	*ND	*ND
Secondary Alpha	*ND	*ND	*ND	*ND	*ND	*ND	*ND
Secondary Alpha	*ND	*ND	*ND	*ND	*ND	*ND	*ND
Secondary Alpha	*ND	*ND	*ND	*ND	*ND	*ND	*ND

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Secondary Beta	*ND	*ND	*ND	*ND	*ND	*ND	*ND
Secondary Beta	*ND	*ND	*ND	*ND	*ND	*ND	*ND
Secondary Beta	*ND	*ND	*ND	*ND	*ND	*ND	*ND
Secondary Beta	*ND	*ND	*ND	*ND	*ND	*ND	*ND

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Tertiary Alpha	0.786	0.37	0.357	0.459	0.36	0.647	0.664
Tertiary Alpha	1.34	1.225	0.963	1.38	1.72	0.539	1.18
Tertiary Alpha	0.458	0.644	0.508	0.474	1.19	0.527	0.147
Tertiary Alpha	0.15	0.269	0.141	0.783	2.72	0.425	0.241

	1.75µg Xa & 4.10µg ATIII	1.0µg PS	1.0µg Heparin	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Tertiary Beta	1.05	0.646	0.125	0.615	0.693	0.479	0.414
Tertiary Beta	1.32	0.452	0.954	2.25	1.22	1.16	0.72
Tertiary Beta	0.619	1.25	0.219	0.372	1.32	0.422	0.071
Tertiary Beta	0.229	0.872	0.114	0.89	1.78	0.498	0.18

P Values for Primary Alpha

Primary Alpha

	1.75µg Xa & 4.10µg ATIII	*1.0µg PS*
Mean	33.0325	18.125
Variance	18.404225	40.16916667
Observations	4	4
t Stat	13.42406433	
P(T<=t) one-tail	0.000446869	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.000893738	
t Critical two-tail	3.182446305	

	1.75µg Xa & 4.10µg ATIII	*1.0µg Heparin*
Mean	33.0325	35.66
Variance	18.404225	34.9224
Observations	4	4
t Stat	-2.600012051	
P(T<=t) one-tail	0.040187493	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.080374986	
t Critical two-tail	3.182446305	

	1.75µg Xa & 4.10µg ATIII	*0.07µg SPM*
Mean	33.0325	34.575
Variance	18.404225	17.41583333
Observations	4	4
t Stat	-2.294822268	
P(T<=t) one-tail	0.052744123	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.105488247	
t Critical two-tail	3.182446305	

	1.75µg Xa & 4.10µg ATIII	*0.7µg SPM*
Mean	33.0325	33.025
Variance	18.404225	8.9225
Observations	4	4
t Stat	0.006638929	
P(T<=t) one-tail	0.497559868	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.995119737	

t Critical two-tail	3.182446305

	1.75µg Xa & 4.10µg ATIII	*0.05µg SPD*
Mean	33.0325	34.225
Variance	18.404225	13.04916667
Observations	4	4
t Stat	-2.450971358	
P(T<=t) one-tail	0.045800097	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.091600195	
t Critical two-tail	3.182446305	

	1.75µg Xa & 4.10µg ATIII	*0.5µg SPD*
Mean	33.0325	28.25
Variance	18.404225	3.136666667
Observations	4	4
t Stat	2.65069069	
P(T<=t) one-tail	0.038474178	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.076948356	
t Critical two-tail	3.182446305	

P Values for Primary Beta

Primary Beta

	1.75µg Xa & 4.10µg ATIII	*1.0µg PS*
Mean	26.2875	25.775
Variance	6.297291667	3.1025
Observations	4	4
t Stat	1.072162632	
P(T<=t) one-tail	0.181113116	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.362226232	
t Critical two-tail	3.182446305	

	1.75µg Xa & 4.10µg ATIII	*1.0µg Heparin*
Mean	26.2875	29.6325
Variance	6.297291667	12.262225
Observations	4	4
t Stat	-5.891510707	
P(T<=t) one-tail	0.004880404	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.009760809	
t Critical two-tail	3.182446305	

	1.75µg Xa & 4.10µg ATIII	*0.07µg SPM*
Mean	26.2875	28.525
Variance	6.297291667	2.53583333
Observations	4	4
t Stat	-3.413189216	
P(T<=t) one-tail	0.021026117	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.042052234	
t Critical two-tail	3.182446305	

	1.75µg Xa & 4.10µg ATIII	*0.7µg SPM*
Mean	26.2875	26.275
Variance	6.297291667	3.4425
Observations	4	4
t Stat	0.012426756	
P(T<=t) one-tail	0.49543267	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.990865341	

t Critical two-tail	3.182446305

	1.75µg Xa & 4.10µg ATIII	*0.05µg SPD*
Mean	26.2875	26.975
Variance	6.297291667	1.20916667
Observations	4	4
t Stat	-0.716383119	
P(T<=t) one-tail	0.262743311	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.525486621	
t Critical two-tail	3.182446305	

	1.75µg Xa & 4.10µg ATIII	*0.5µg SPD*
Mean	26.2875	22.925
Variance	6.297291667	2.9825
Observations	4	4
t Stat	1.636243759	
P(T<=t) one-tail	0.100153875	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.200307749	
t Critical two-tail	3.182446305	

P Values for Tertiary Alpha

Tertiary Alpha

	1.75µg Xa & 4.10µg ATIII	*1.0µg PS*
Mean	0.6835	0.627
Variance	0.258990333	0.184035333
Observations	4	4
t Stat	0.41514482	
P(T<=t) one-tail	0.35296989	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.705939779	
t Critical two-tail	3.182446305	

	1.75µg Xa & 4.10µg ATIII	*1.0µg Heparin*
Mean	0.6835	0.49225
Variance	0.258990333	0.12117425
Observations	4	4
t Stat	1.551052084	
P(T<=t) one-tail	0.109340383	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.218680766	
t Critical two-tail	3.182446305	

	1.75µg Xa & 4.10µg ATIII	*0.07µg SPM*
Mean	0.6835	0.774
Variance	0.258990333	0.185514
Observations	4	4
t Stat	-0.454057344	
P(T<=t) one-tail	0.34031223	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.68062446	
t Critical two-tail	3.182446305	

	1.75µg Xa & 4.10µg ATIII	*0.7µg SPM*
Mean	0.6835	1.4975
Variance	0.258990333	0.977491667
Observations	4	4
t Stat	-1.284878464	
P(T<=t) one-tail	0.144527018	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.289054036	

t Critical two-tail	3.182446305

	1.75µg Xa & 4.10µg ATIII	*0.05µg SPD*
Mean	0.6835	0.5345
Variance	0.258990333	0.008241
Observations	4	4
t Stat	0.638977112	
P(T<=t) one-tail	0.284140028	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.568280056	
t Critical two-tail	3.182446305	

	1.75µg Xa & 4.10µg ATIII	*0.5µg SPD*
Mean	0.6835	0.558
Variance	0.258990333	0.22251
Observations	4	4
t Stat	1.513724567	
P(T<=t) one-tail	0.113657605	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.227315209	
t Critical two-tail	3.182446305	

P Values for Tertiary Beta

Tertiary Beta

	1.75µg Xa & 4.10µg ATIII	*1.0µg PS*
Mean	0.8045	0.805
Variance	0.230540333	0.117468
Observations	4	4
t Stat	-0.001317521	
P(T<=t) one-tail	0.499515742	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.999031484	
t Critical two-tail	3.182446305	

	1.75µg Xa & 4.10µg ATIII	*1.0µg Heparin*
Mean	0.8045	0.353
Variance	0.230540333	0.162754
Observations	4	4
t Stat	2.653599311	
P(T<=t) one-tail	0.03837861	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.07675722	
t Critical two-tail	3.182446305	

	1.75µg Xa & 4.10µg ATIII	*0.07µg SPM*
Mean	0.8045	1.03175
Variance	0.230540333	0.70439225
Observations	4	4
t Stat	-0.678664488	
P(T<=t) one-tail	0.273007483	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.546014966	
t Critical two-tail	3.182446305	

	1.75µg Xa & 4.10µg ATIII	*0.7µg SPM*
Mean	0.8045	1.25325
Variance	0.230540333	0.19896892
Observations	4	4
t Stat	-1.041227028	
P(T<=t) one-tail	0.187151948	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.374303896	

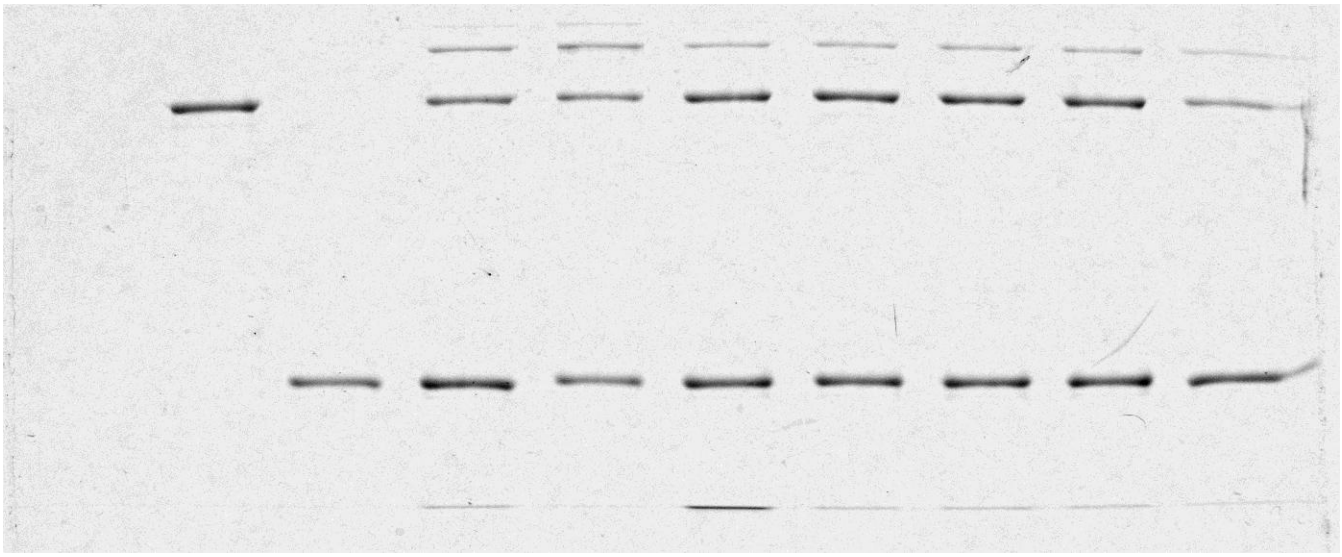
t Critical two-tail	3.182446305

	1.75µg Xa & 4.10µg ATIII	*0.05µg SPD*
Mean	0.8045	0.63975
Variance	0.230540333	0.12133625
Observations	4	4
t Stat	0.958885578	
P(T<=t) one-tail	0.20417735	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.4083547	
t Critical two-tail	3.182446305	

	1.75µg Xa & 4.10µg ATIII	*0.5µg SPD*
Mean	0.8045	0.34625
Variance	0.230540333	0.08256025
Observations	4	4
t Stat	3.330132065	
P(T<=t) one-tail	0.022357402	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.044714803	
t Critical two-tail	3.182446305	

XIa

The Effects of Heparin, Protamine Sulfate, Spermine, and Spermidine on the Interaction between Factor XIa and Anti-Thrombin III



1. 5µL Molecular Weight Markers+ 13µL Tris/NaCl pH8 + 5µL 5X Sample Buffer
2. 2.0µL (0.762µg) FXIa + 13µL Tris/NaCl pH8 + 5µL 5X Sample Buffer
3. 2.0µL (4.10µg) ATIII + 13µL Tris/NaCl pH8 + 5µL 5X Sample Buffer
4. 2.0µL (0.762µg) FXIa + 2.0µL (4.10µg) ATIII + 11µL Tris/NaCl pH8 + 5µL 5X Sample Buffer
5. [(2.0µL (4.10µg) ATIII + 1µg (3µL) Heparin) 15’ at RT, + 1.0µL (0.762µg) FXIa + 8µL Tris/NaCl pH8] 60’ at RT, + 5µL 5X Sample Buffer]
6. [(2.0µL (4.10µg) ATIII + 1µg (3µL) Protamine Sulfate) 15’ at RT, + 2.0µL (0.762µg) FXIa + 8µL Tris/NaCl pH8] 60’ at RT, + 5µL 5X Sample Buffer]
7. [(2.0µL (4.10µg) ATIII + 0.07µg (3µL) Spermine) 15’ at RT, + 2.0µL (0.762µg) FXIa + 8µL Tris/NaCl pH8] 60’ at RT, + 5µL 5X Sample Buffer]
8. [(2.0µL (4.10µg) ATIII + 0.7µg (3µL) Spermine) 15’ at RT, + 2.0µL (0.762µg) FXIa + 8µL Tris/NaCl pH8] 60’ at RT, + 5µL 5X Sample Buffer]
9. [(2.0µL (4.10µg) ATIII + 0.05µg (3µL) Spermidine) 15’ at RT, + 2.0µL (0.762µg) FXIa + 8µL Tris/NaCl pH8] 60’ at RT, + 5µL 5X Sample Buffer]
10. [(2.0µL (4.10µg) ATIII + 0.5µg (3µL) Spermidine) 15’ at RT, + 2.0µL (0.762 µg) FXIa + 8µL Tris/NaCl pH8] 60’ at RT, + 5µL 5X Sample Buffer]

Pictures of Each Gel:
Figure A

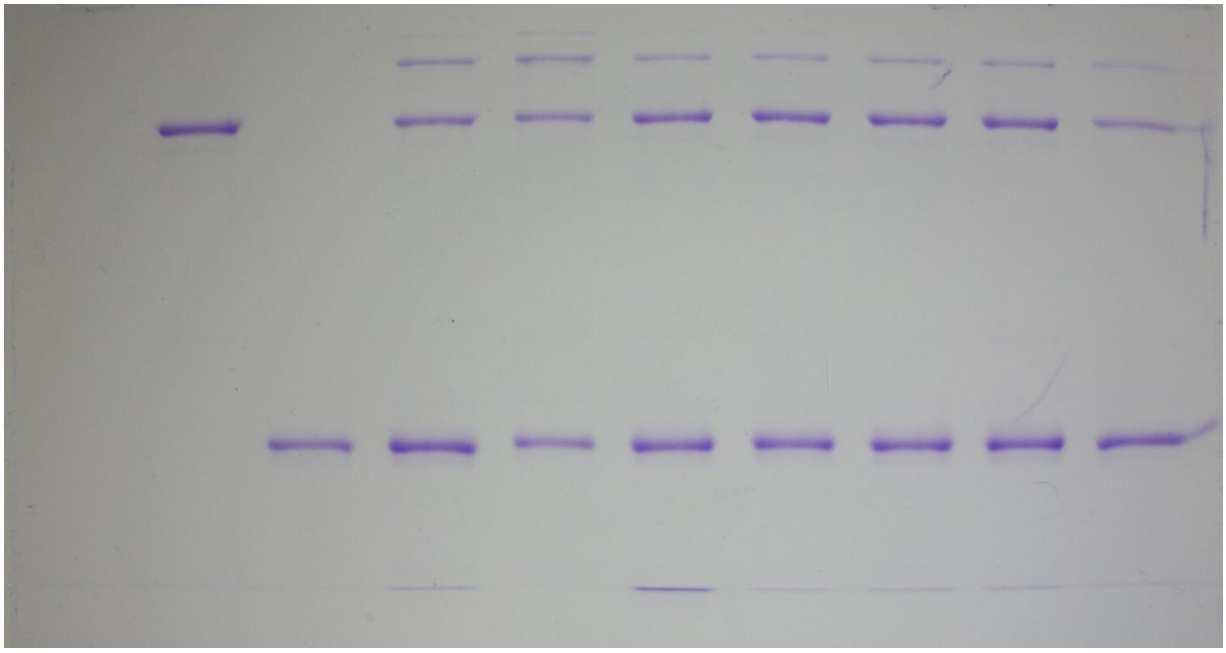
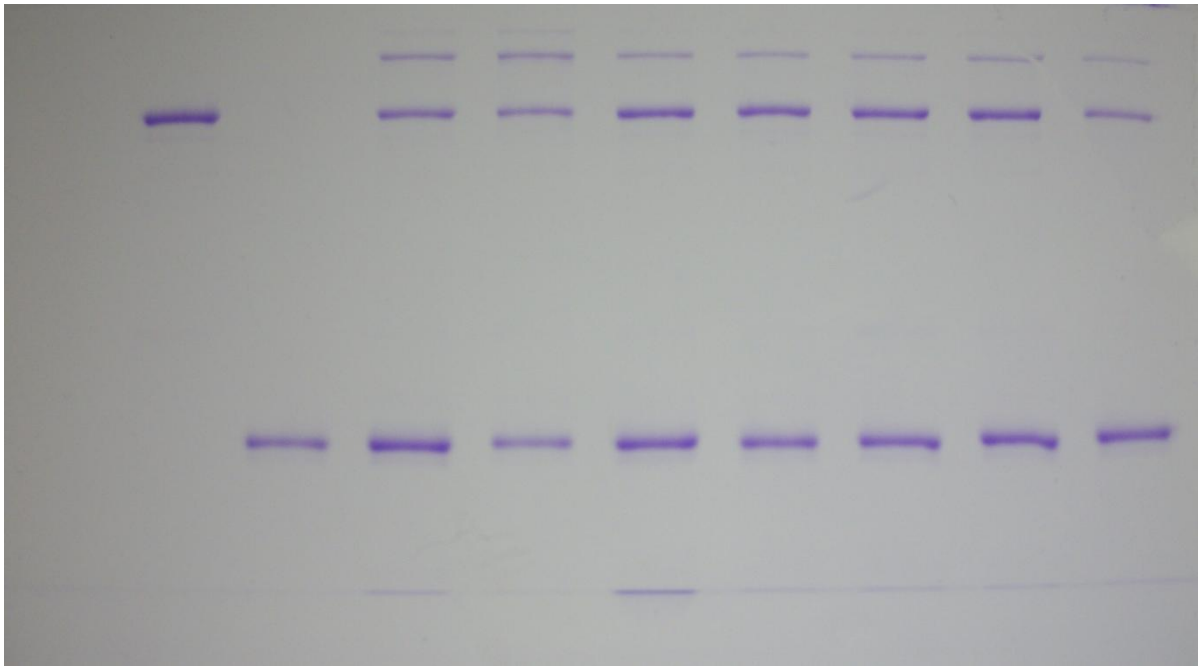


Figure B



Gel 1 analysis will be first, followed by Gel 2,3, and 4, analysis, then a summary.

Table 1: Overall Protein Analysis Per Lane

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
[XIa-$(ATIII)_2$]	0.9	2.4	*ND	*ND	*ND	*ND	*ND
[XIa-ATIII]	12.1	19.5	7.1	8.1	8.7	7.2	5
XIa	29.9	31.9	35.2	41.5	40	40	25.2
ATIII	52.8	45.4	43.6	47.4	47.6	49.5	67.2
Solvent Front	4.3	0.8	14	3.1	3.7	3.4	2.6

*ND= Not Detected

Standard Deviation	20.04912043	Mean	23.3366667	Standard Error	3.38892

Figure C: Graphical Representation of Table 1

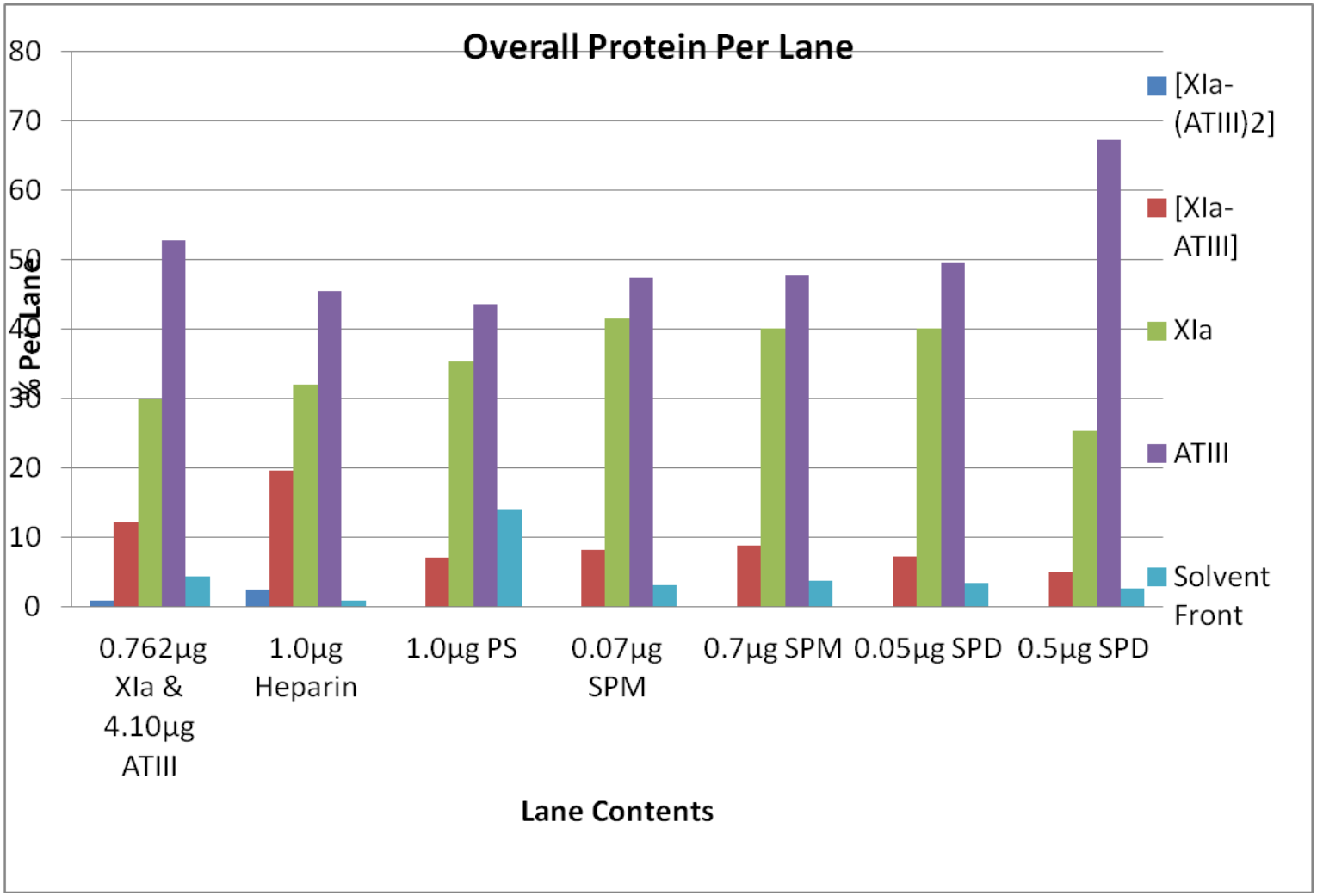


Table 2: [XIa-$(ATIII)_2$] Analysis Per Lane

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
[XIa-$(ATIII)_2$]	0.9	2.4	*ND	*ND	*ND	*ND	*ND

*ND=Not Detected

Figure D: Graphical Representation of Table 2

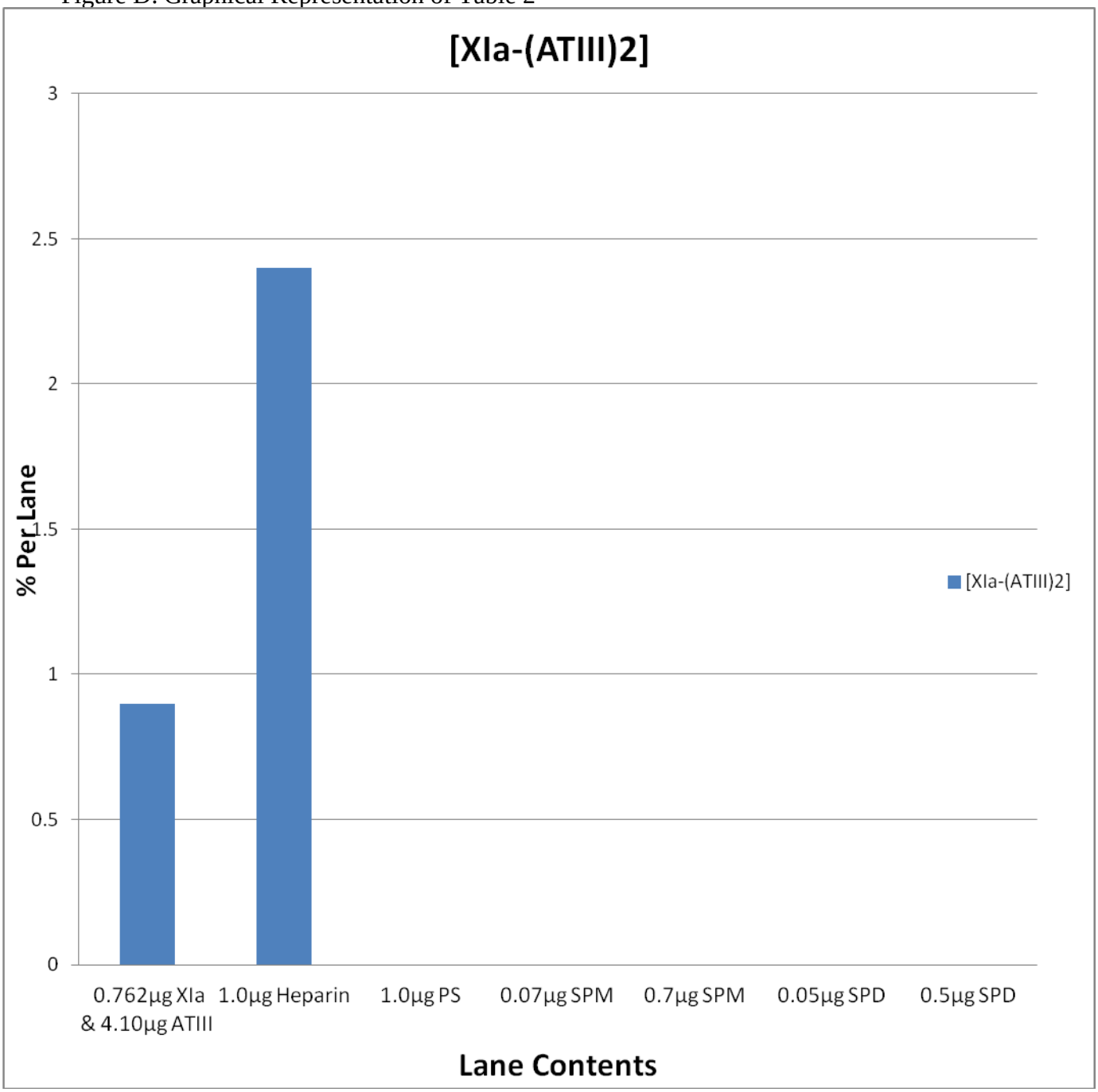


Table 3: [XIa-ATIII] Analysis Per Lane

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
[XIa-ATIII]	12.1	19.5	7.1	8.1	8.7	7.2	5

Figure E: Graphical Representation of Table 3

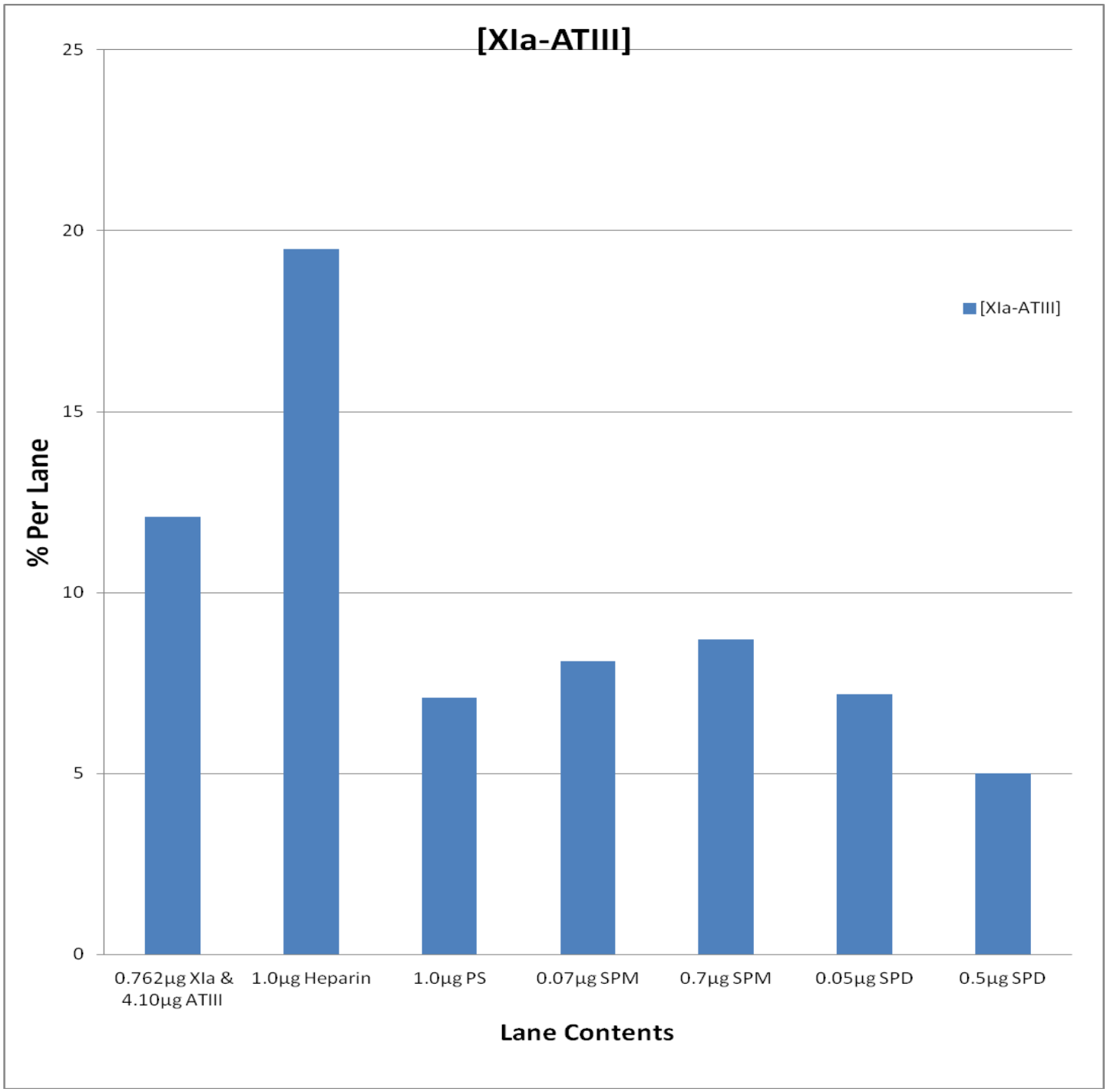


Table 4: XIa Per Lane Analysis

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
XIa	29.9	31.9	35.2	41.5	40	40	25.2

Figure F: Graphical Representation of Table 4

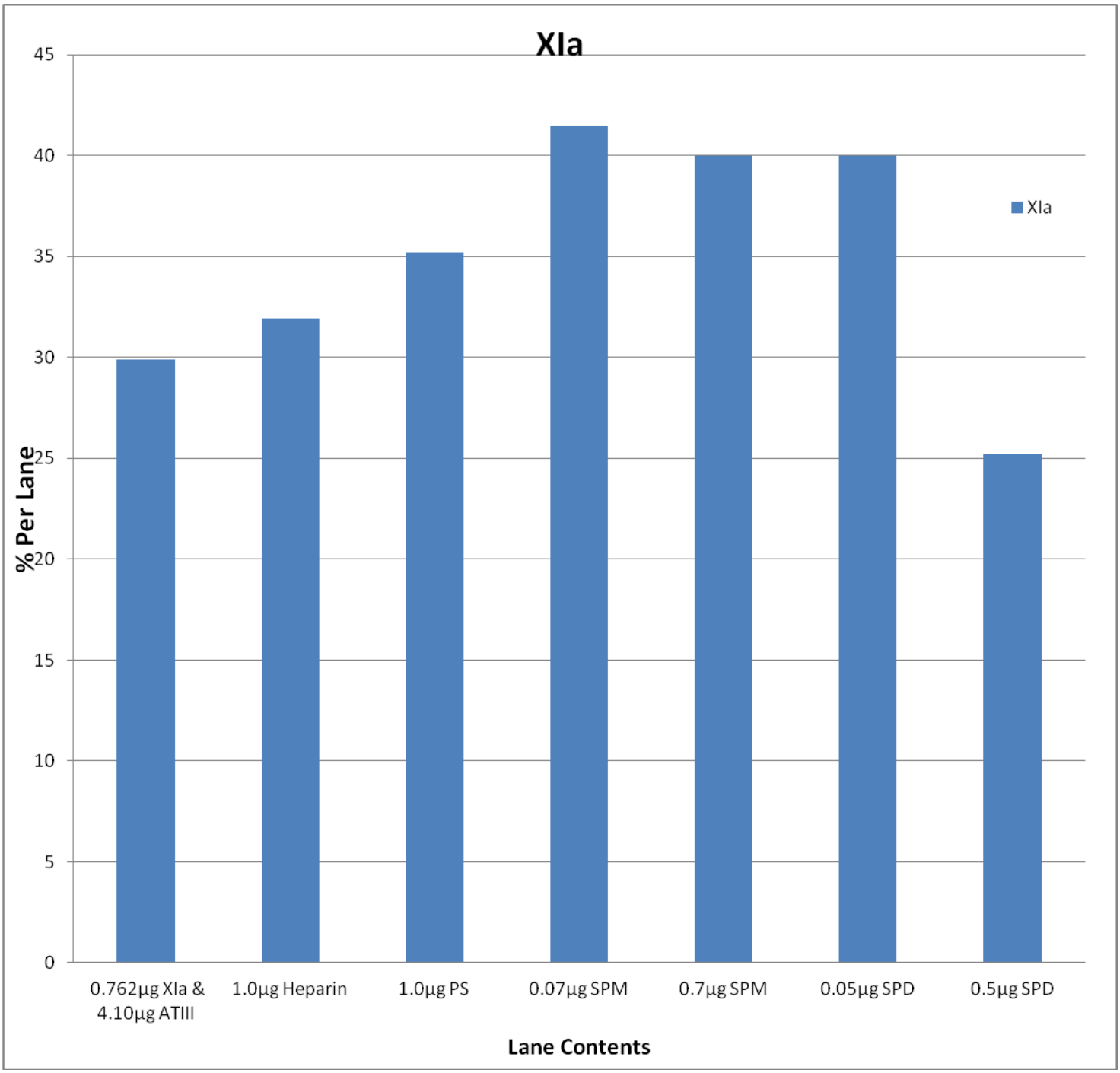


Table 5: ATIII Per Lane Analysis

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIII	52.8	45.4	43.6	47.4	47.6	49.5	67.2

Figure G: Graphical Representation of Table 5

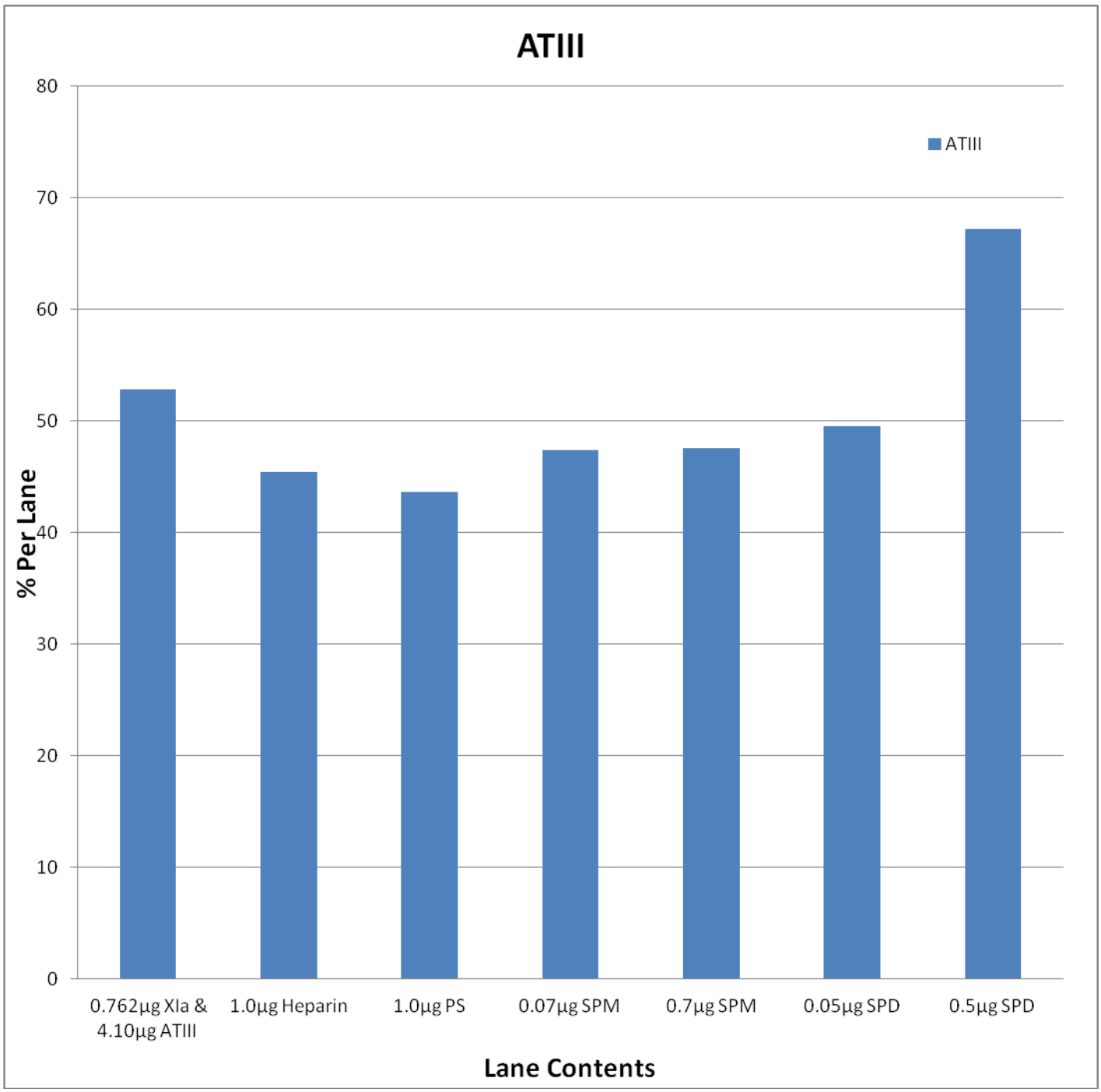


Table 6: Solvent Front Analysis Per Lane

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Solvent Front	4.3	0.8	14	3.1	3.7	3.4	2.6

Figure H: Graphical Representation of Table 6

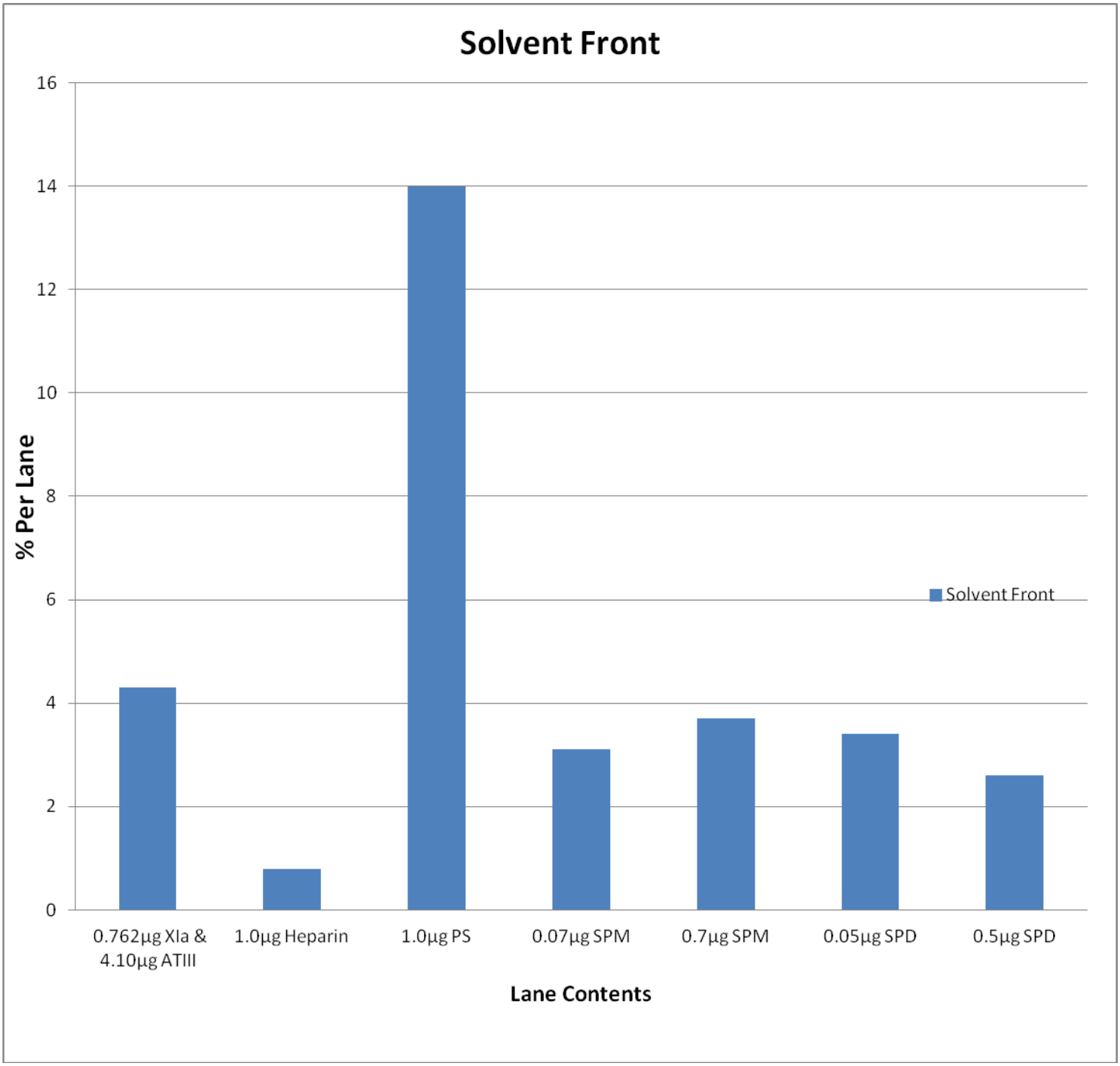


Table 7: Overall Protein Analysis Per Lane

	0.762µg Xla & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
[Xla-$(ATIII)_2$]	0.4	1.7	*ND	*ND	*ND	*ND	*ND
[Xla-ATIII]	11.6	19.4	6.7	7.6	7.5	6.6	4.2
Xla	30.3	33.9	35.5	42.1	40.6	40.6	27.3
ATIII	53.5	44.1	45.1	47	48.6	50.3	65.9
Solvent Front	4.2	0.9	12.6	3.3	3.4	2.5	2.6

*ND= Not Detected

Standard Deviation	20.37603957	Mean	23.3333333	Standard Error	3.444179

Figure I: Graphical Representation of Table 7

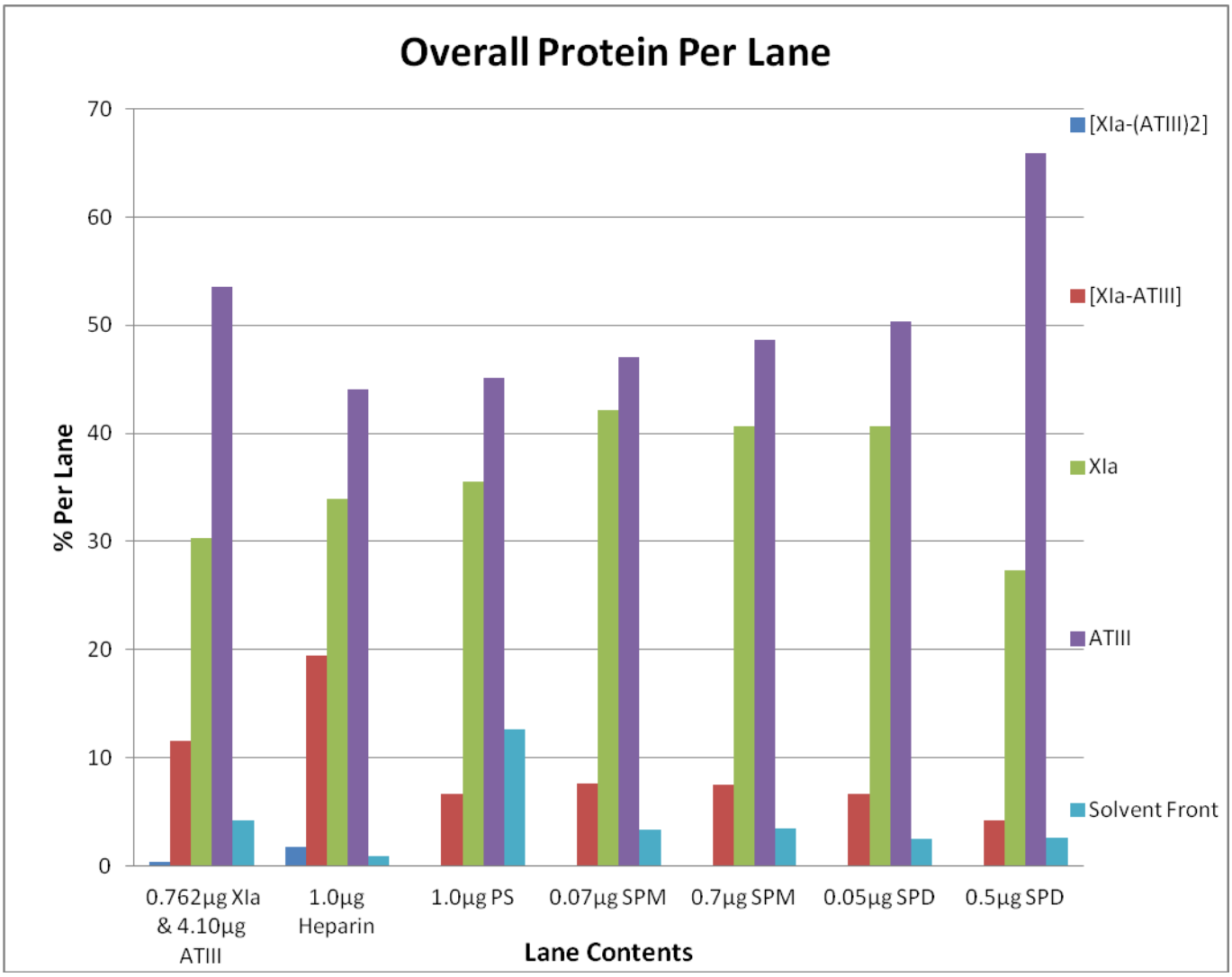


Table 8: [XIa-$(ATIII)_2$] Analysis Per Lane

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
[XIa-(ATIII)2]	0.4	1.7	*ND	*ND	*ND	*ND	*ND

*ND= Not Detected

Figure J: Graphical Representation of Table 8

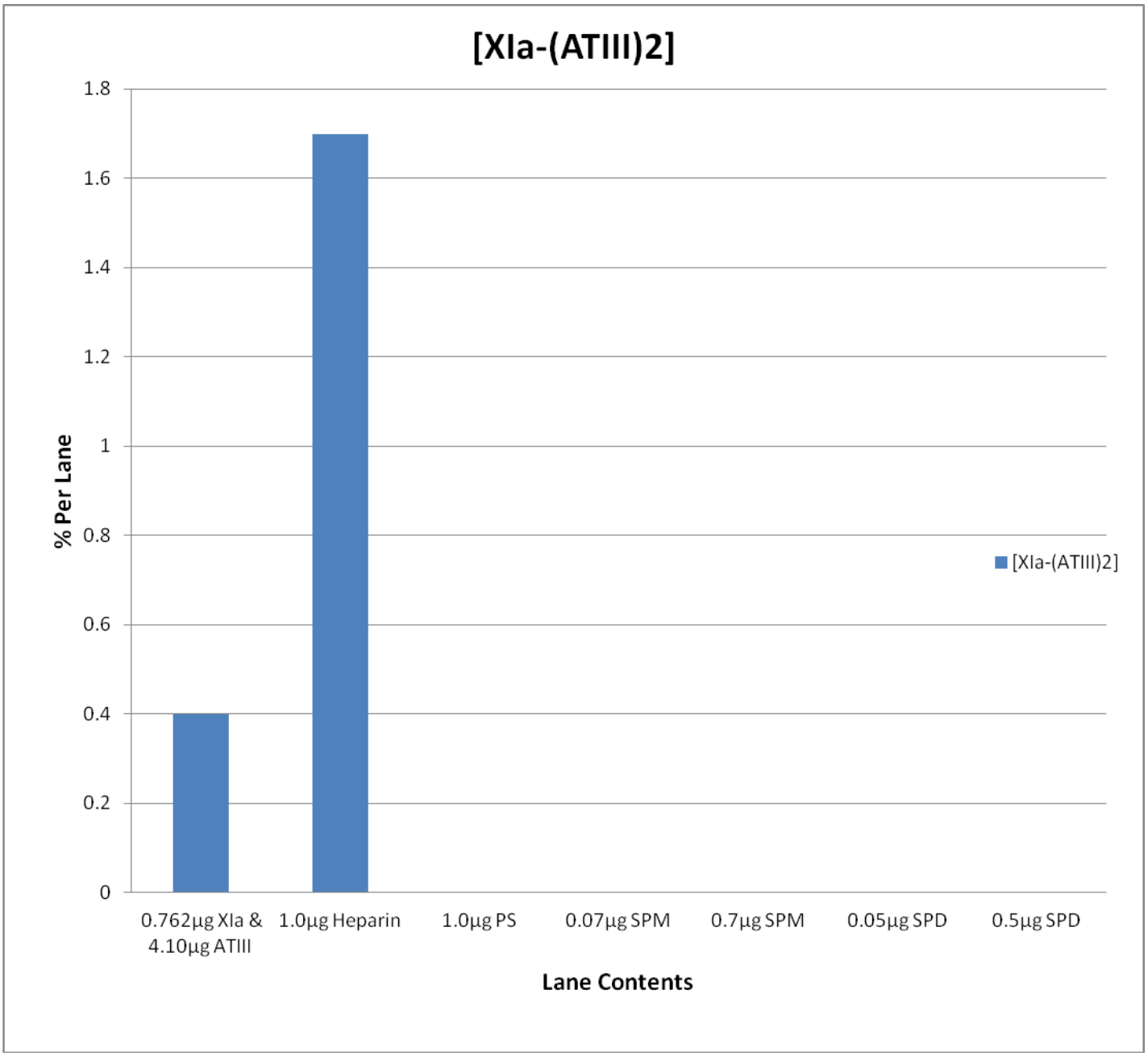


Table 9: [XIa-ATIII] Analysis Per Lane

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
[XIa-ATIII]	11.6	19.4	6.7	7.6	7.5	6.6	4.2

Figure K: Graphical Representation of Table 9

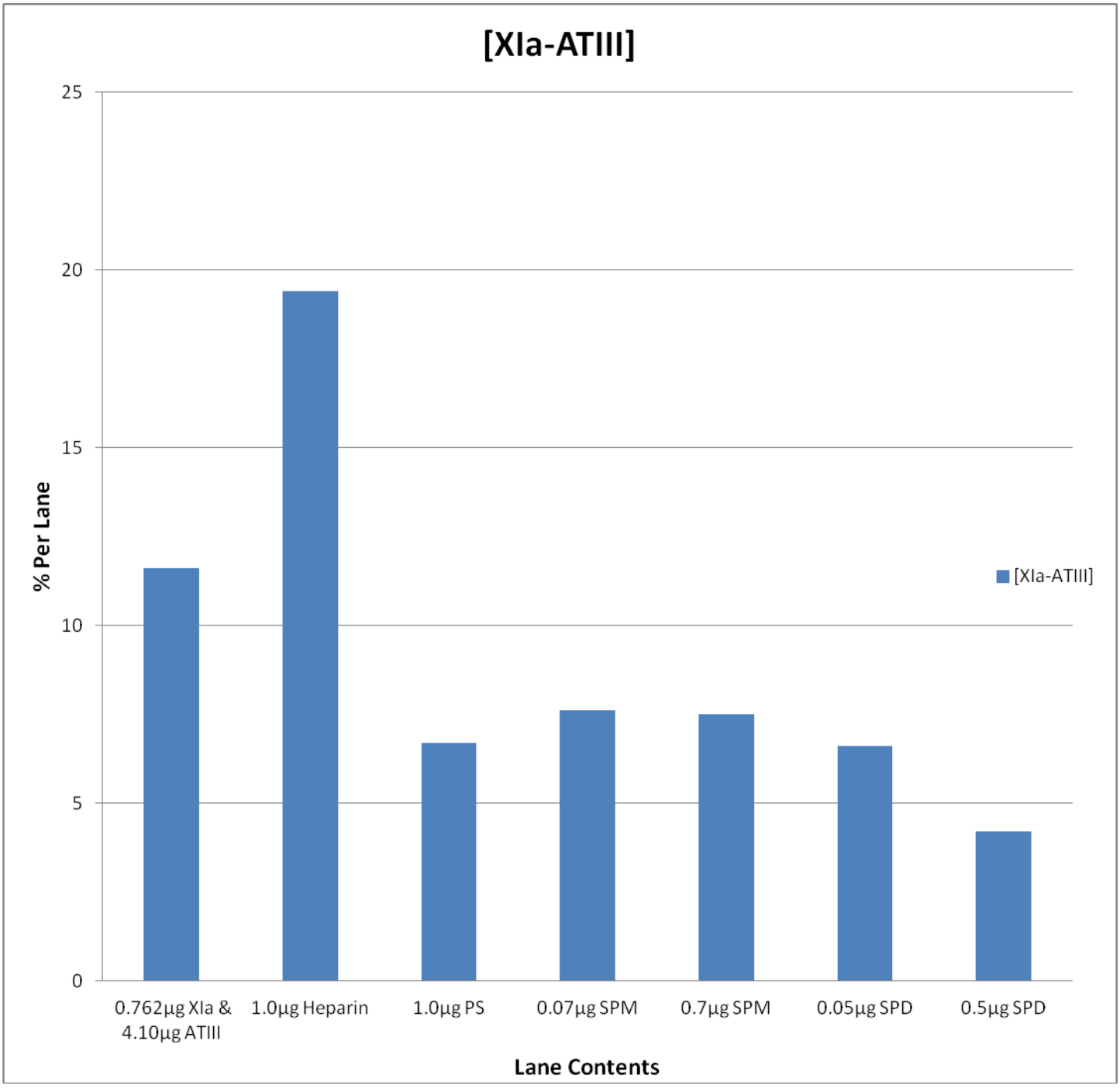


Table 10: XIa Analysis Per Lane

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
XIa	30.3	33.9	35.5	42.1	40.6	40.6	27.3

Figure L: Graphical Representation of Table 10

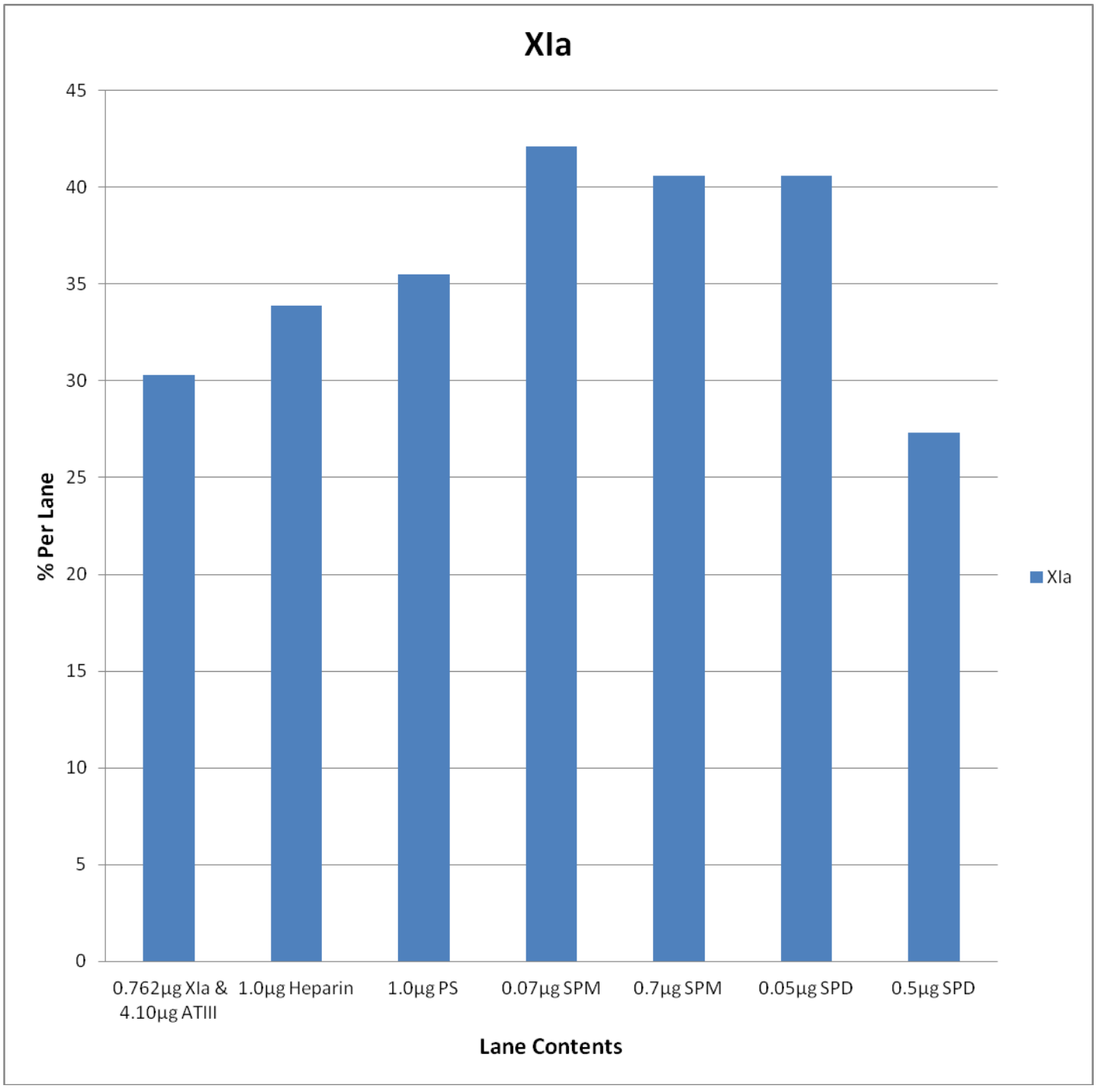


Table 11 ATIII Analysis Per Lane

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIII	53.5	44.1	45.1	47	48.6	50.3	65.9

Figure M: Graphical Representation of Table 11

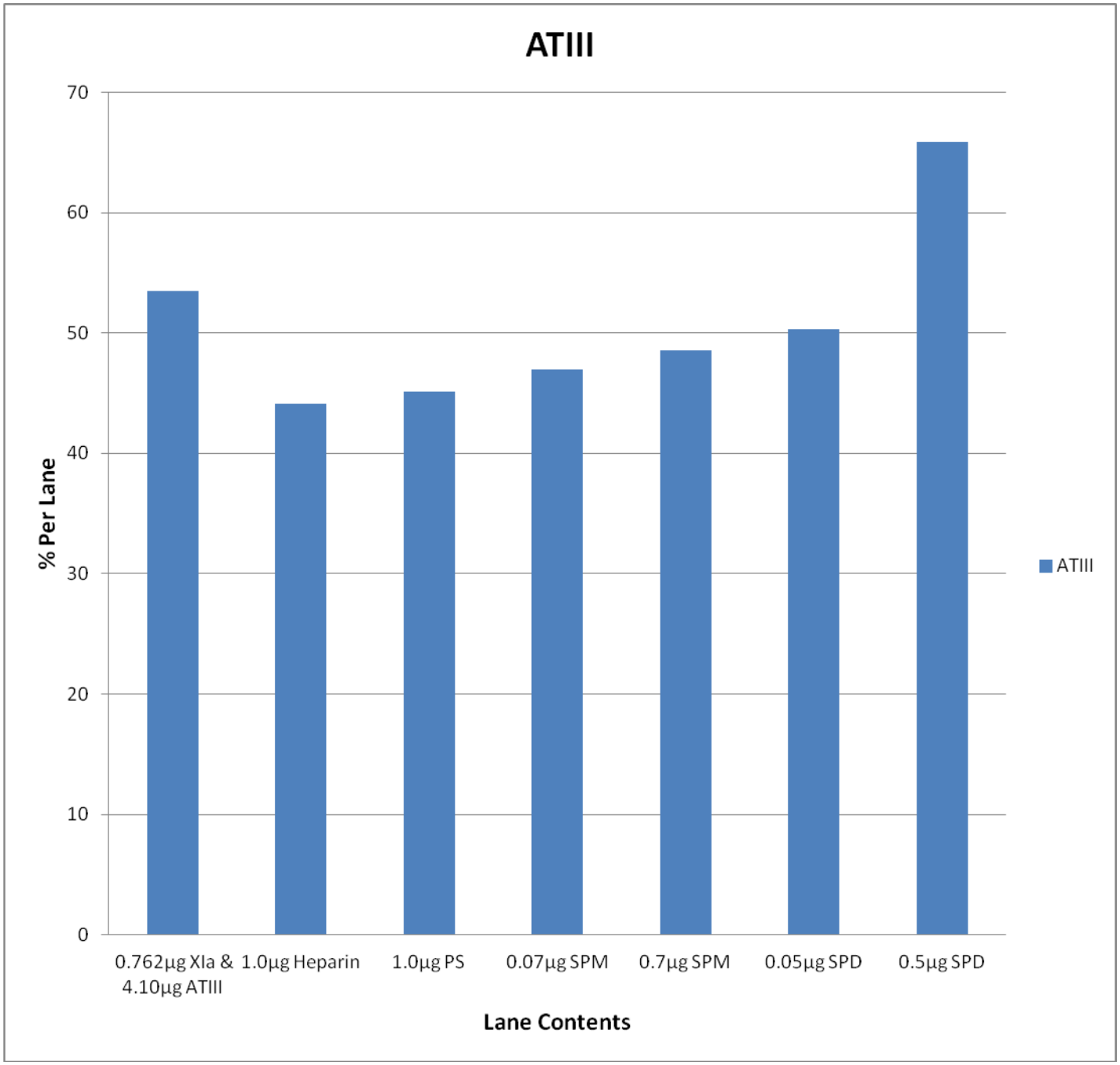


Table 12: Solvent Front Analysis Per Lane

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Solvent Front	4.2	0.9	12.6	3.3	3.4	2.5	2.6

Figure N: Graphical Representation of Table 12

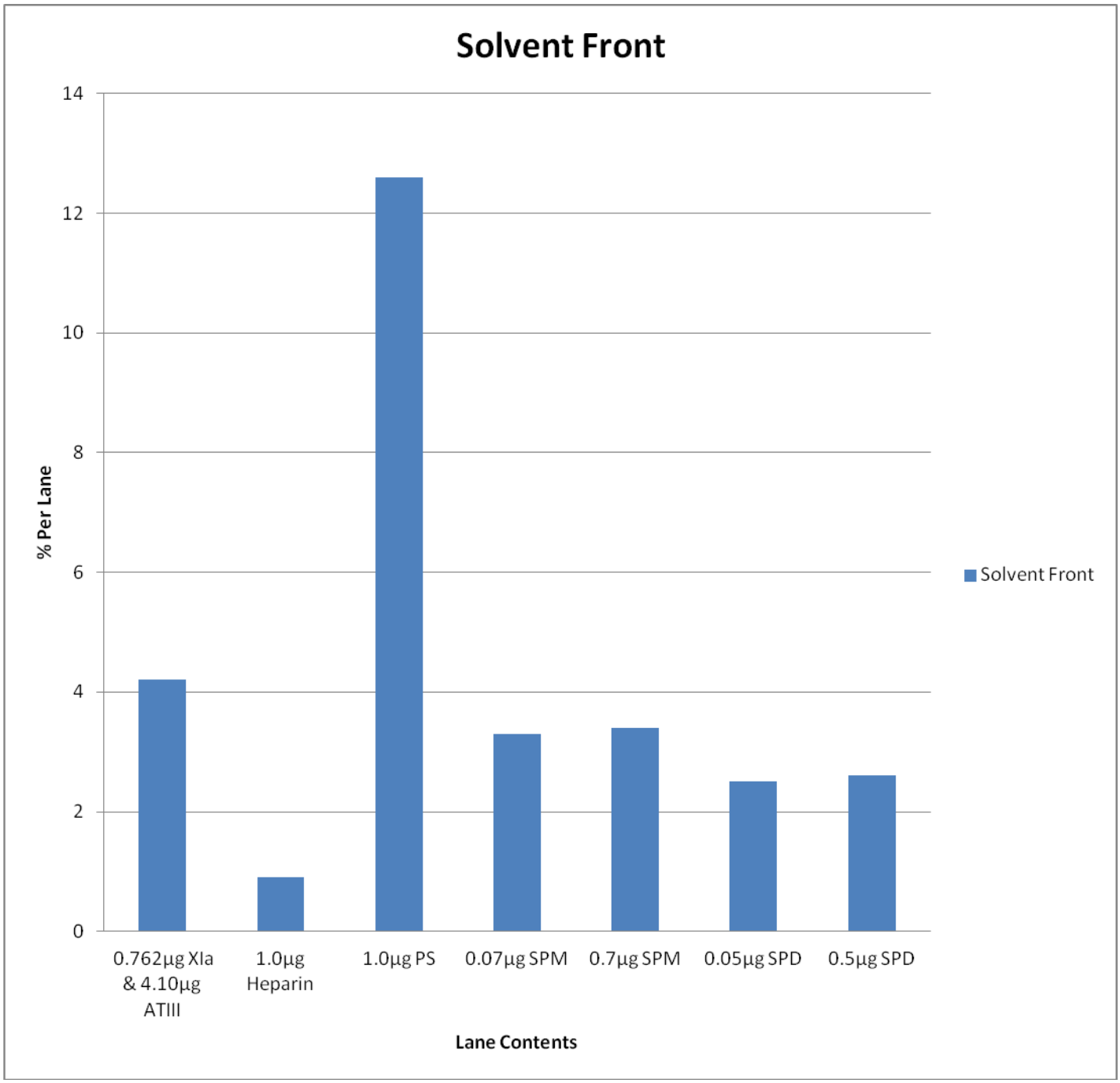


Figure O: Gel 3

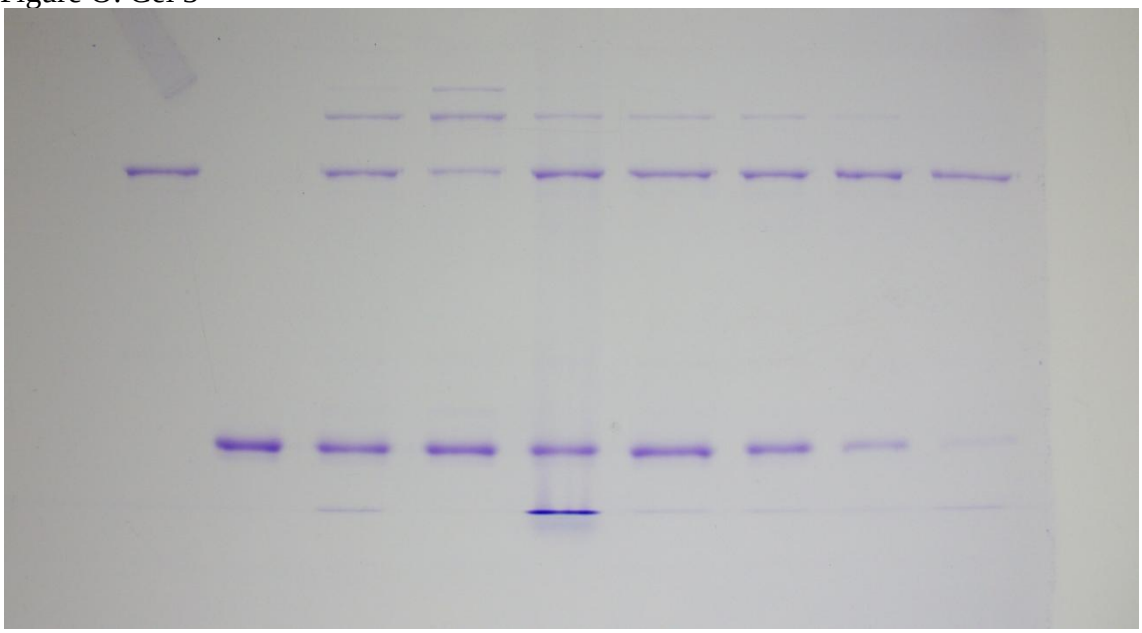
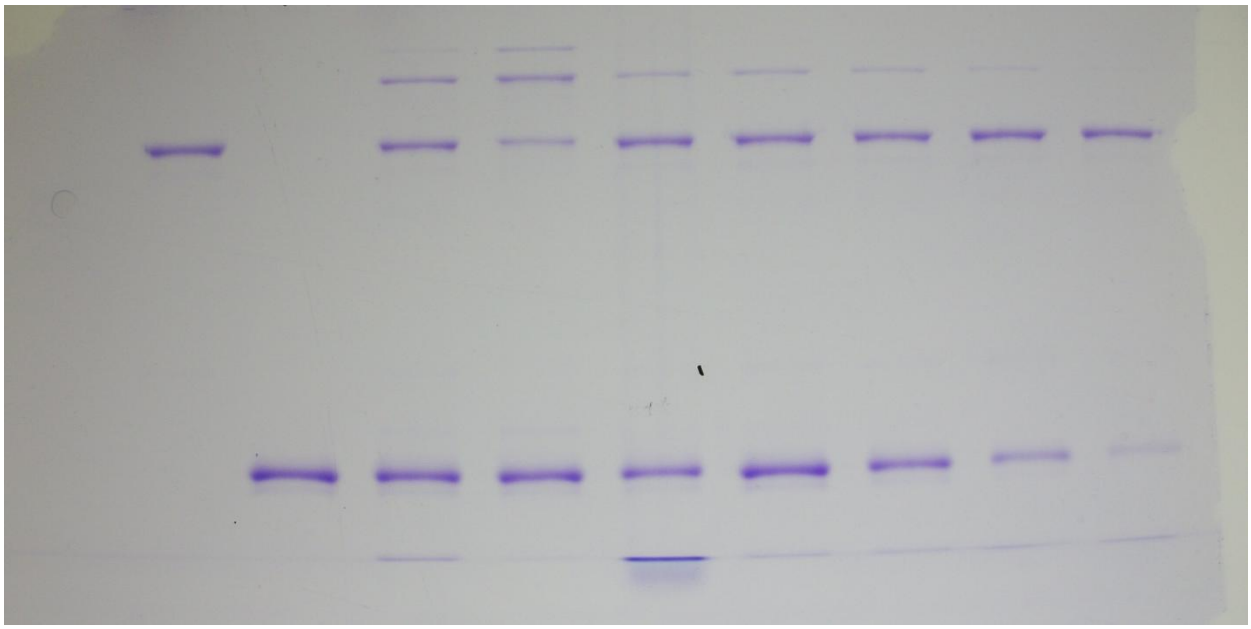


Figure P; Gel 4



Gel 3

Table 12: Overall Protein Analysis Per Lane

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
[XIa-$(ATIII)_2$]	0.6	4.8	*ND	*ND	*ND	*ND	*ND
[XIa-ATIII]	11	16.6	4.1	4.8	4.4	1.7	1.6
XIa	28.4	10.8	31.2	32.8	41.1	66.9	81.1
ATIII	54.3	67.4	37	59.9	51.8	28.9	7.9
Solvent Front	5.8	0.3	27.7	2.5	2.6	2.4	9.4

*ND= Not Detected

Standard Deviation	24.07774668	Mean	23.3266667	Standard Error	4.069882

Figure Q: Graphical Representation of Table 12

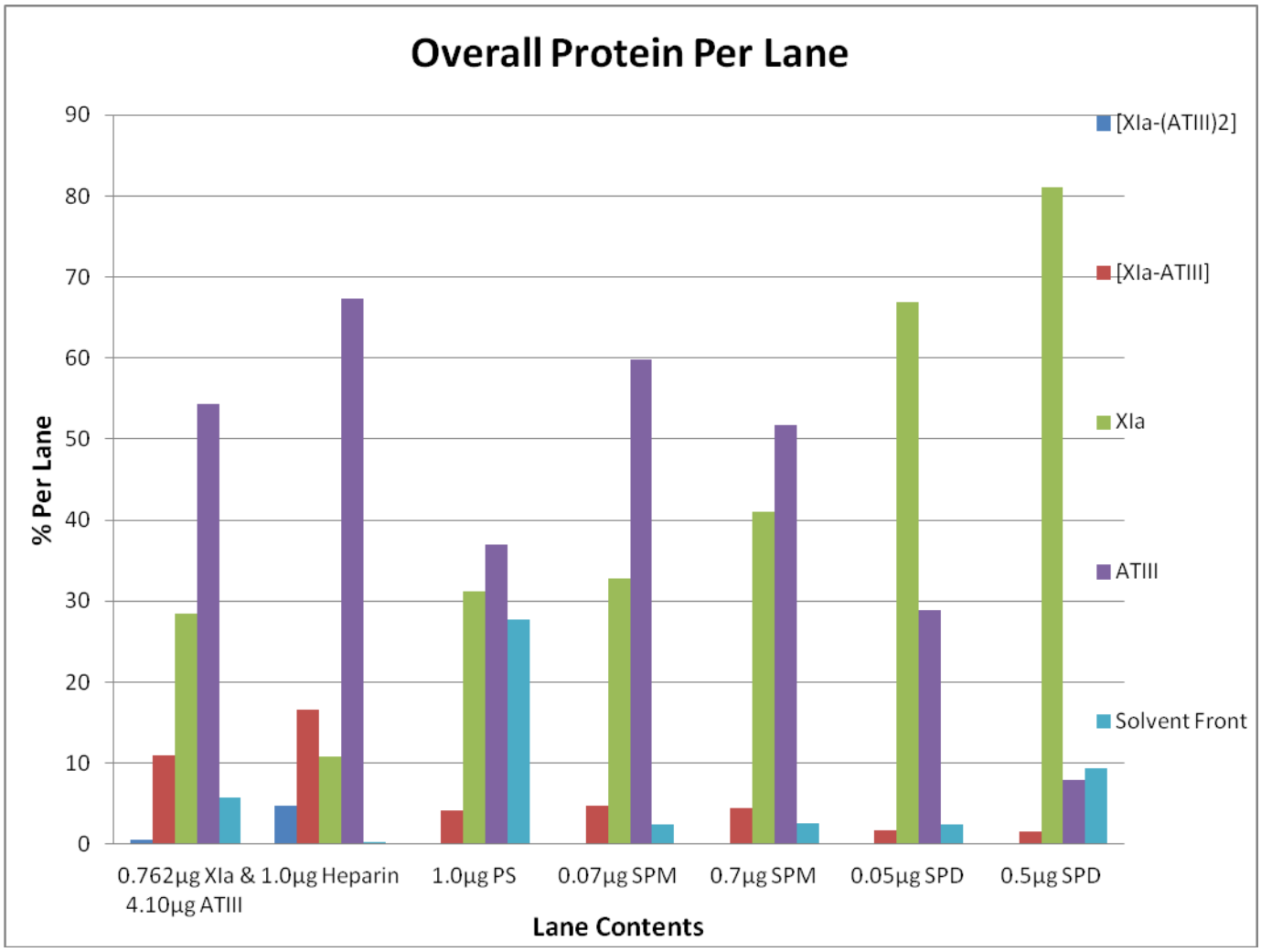


Table 13: [XIa-$(ATIII)_2$] Analysis Per Lane

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD
[XIa-$(ATIII)_2$]	0.6	4.8	*ND	*ND	*ND	*ND

*ND= Not Detected

Figure R: Graphical Representation of Table 13

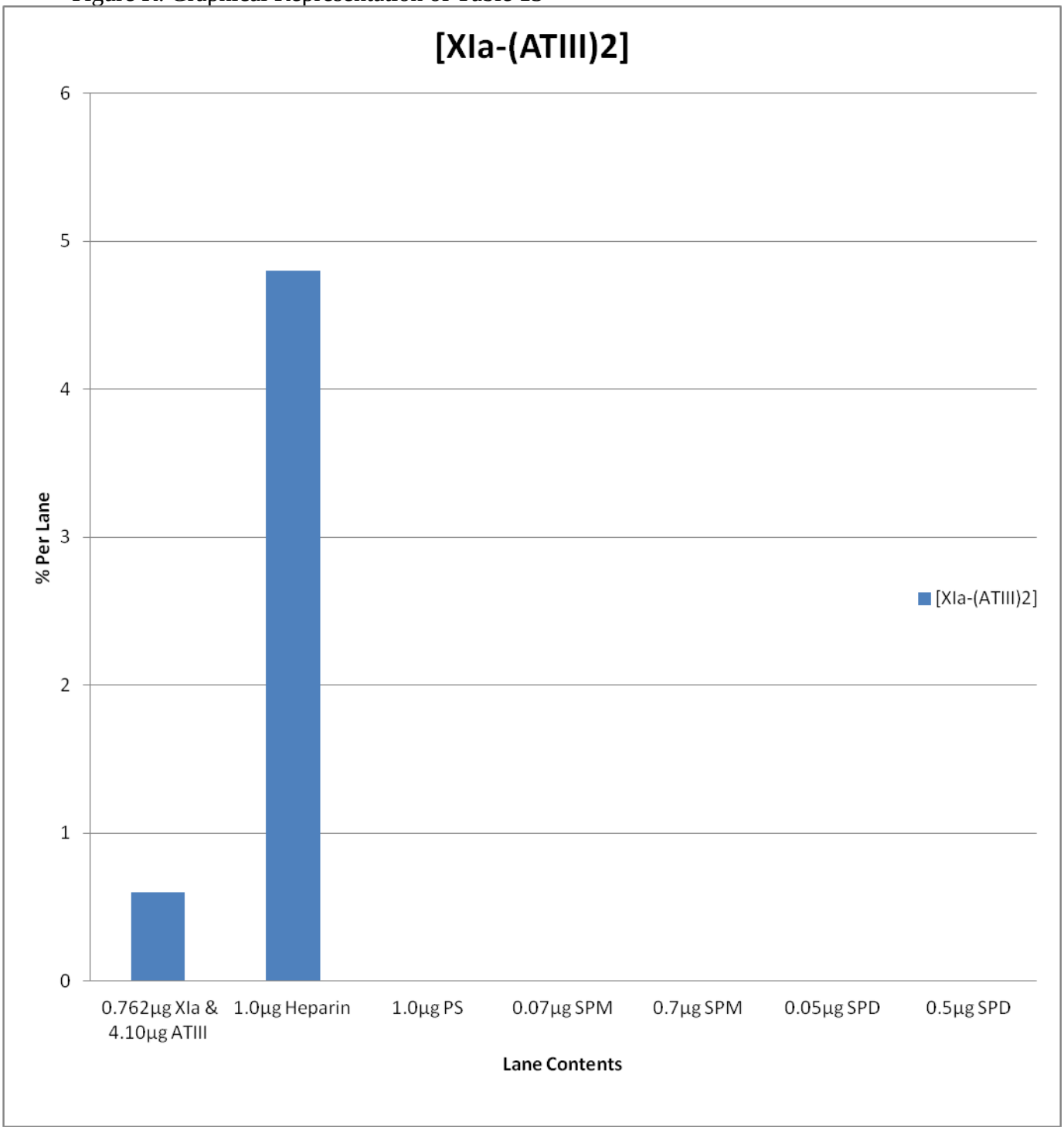


Table 14: [XIa-ATIII] Analysis Per Lane

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
[XIa-ATIII]	11	16.6	4.1	4.8	4.4	1.7	1.6

Figure S: Graphical Representation of Table 14

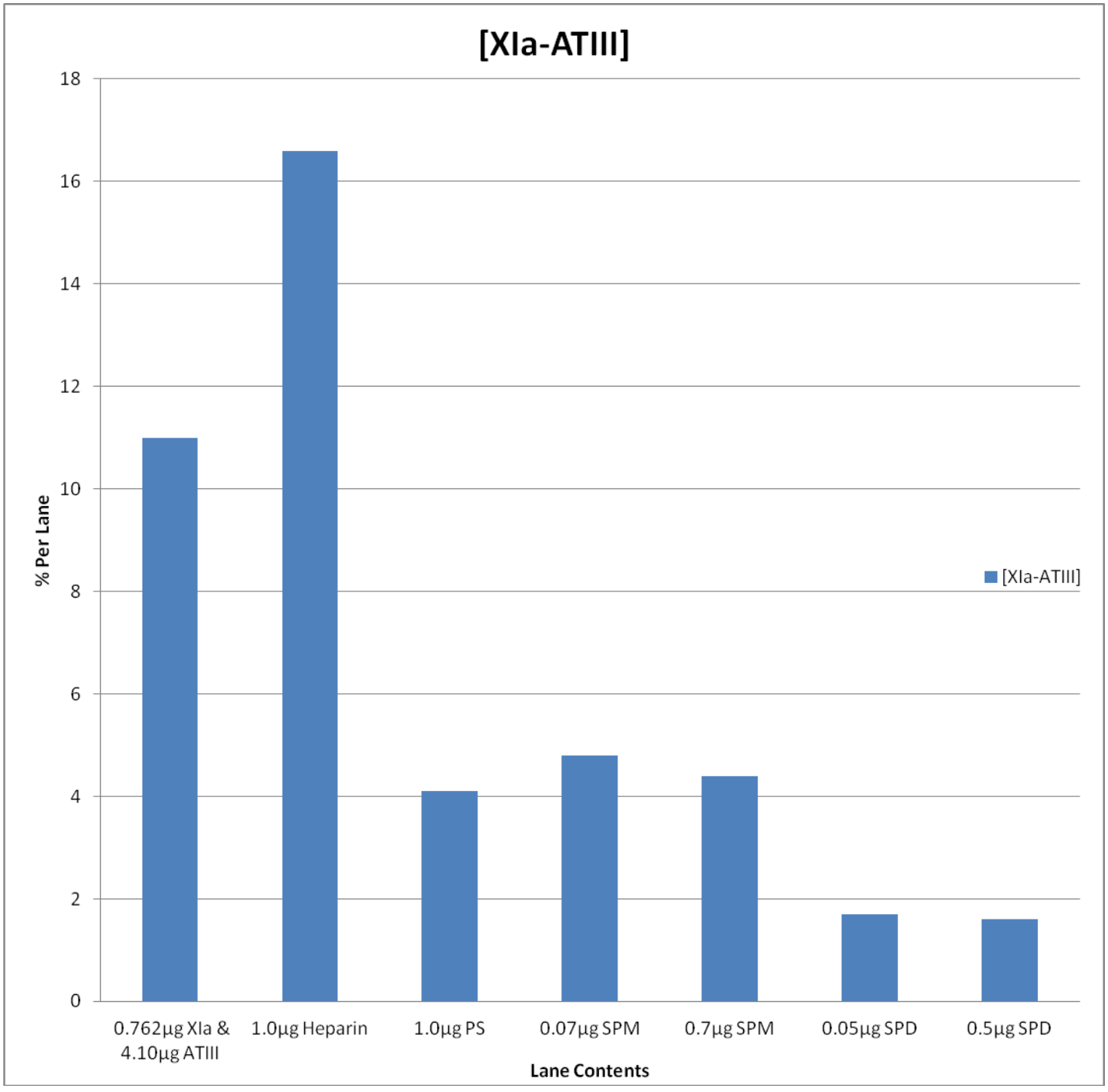


Table 15: XIa Analysis Per Lane

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
XIa	28.4	10.8	31.2	32.8	41.1	66.9	81.1

Figure T: Graphical Representation of Table 15

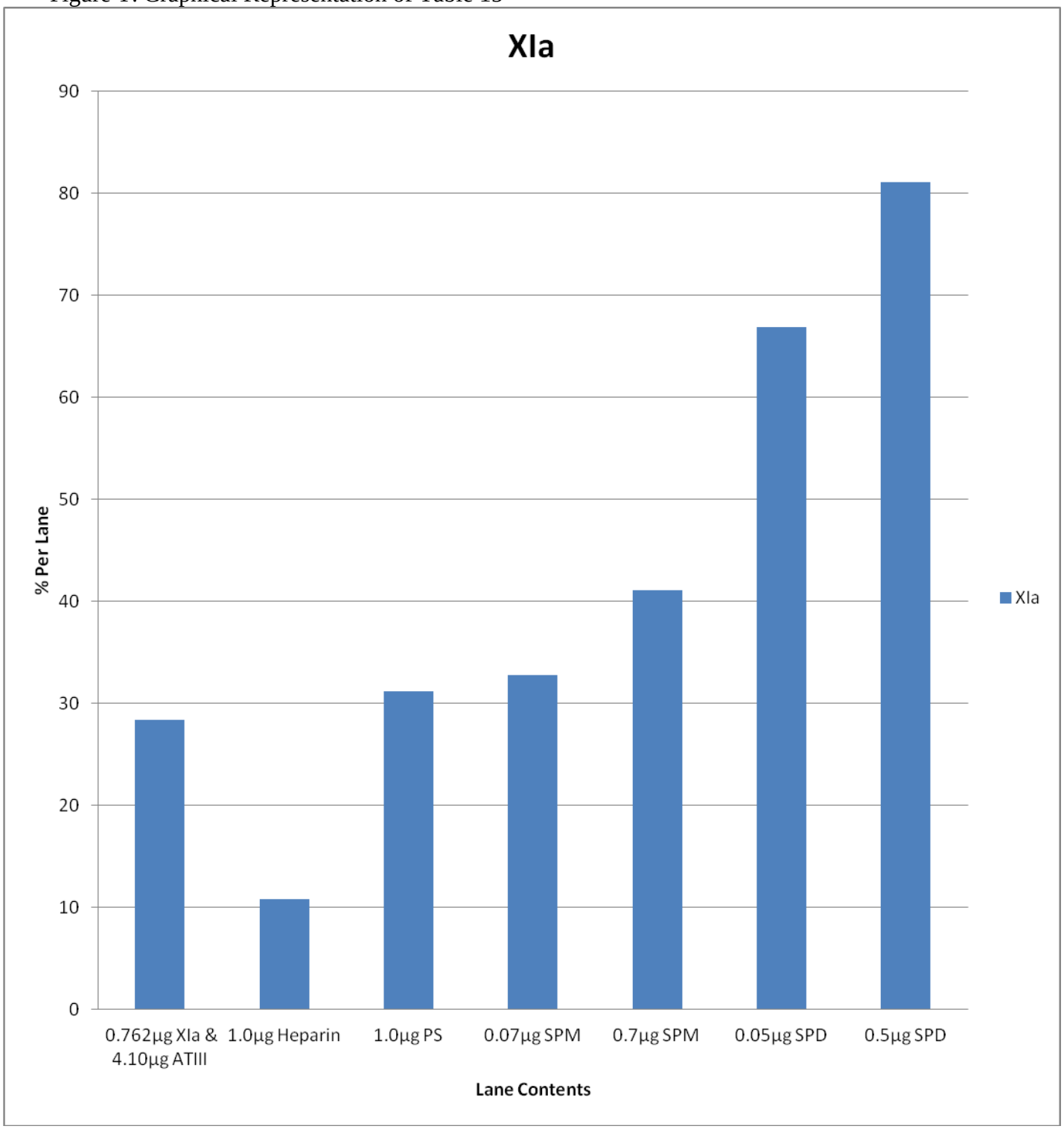


Table 16: ATIII Analysis Per Lane

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIII	54.3	67.4	37	59.9	51.8	28.9	7.9

Figure U: Graphical Representation of Table 16

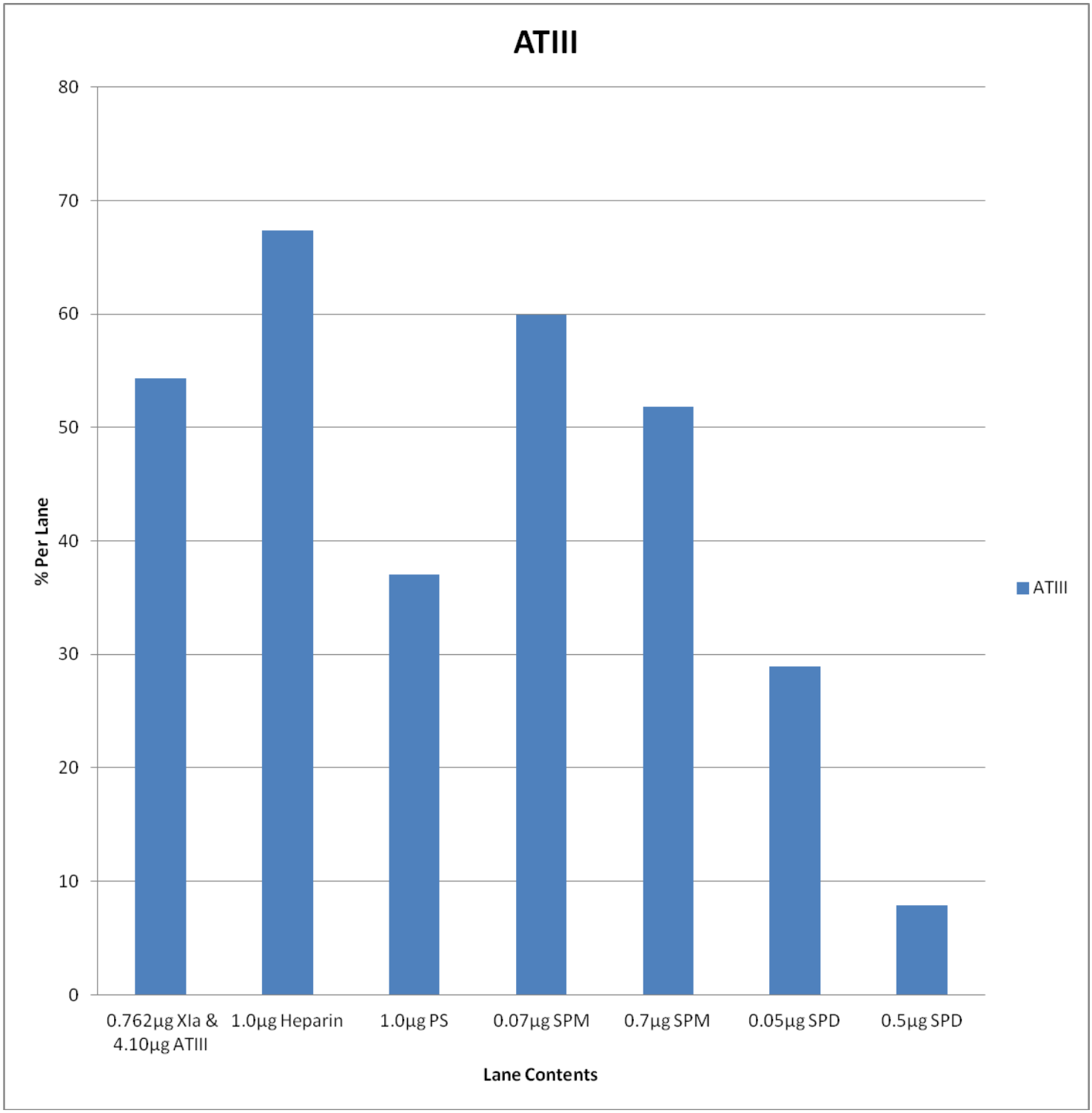
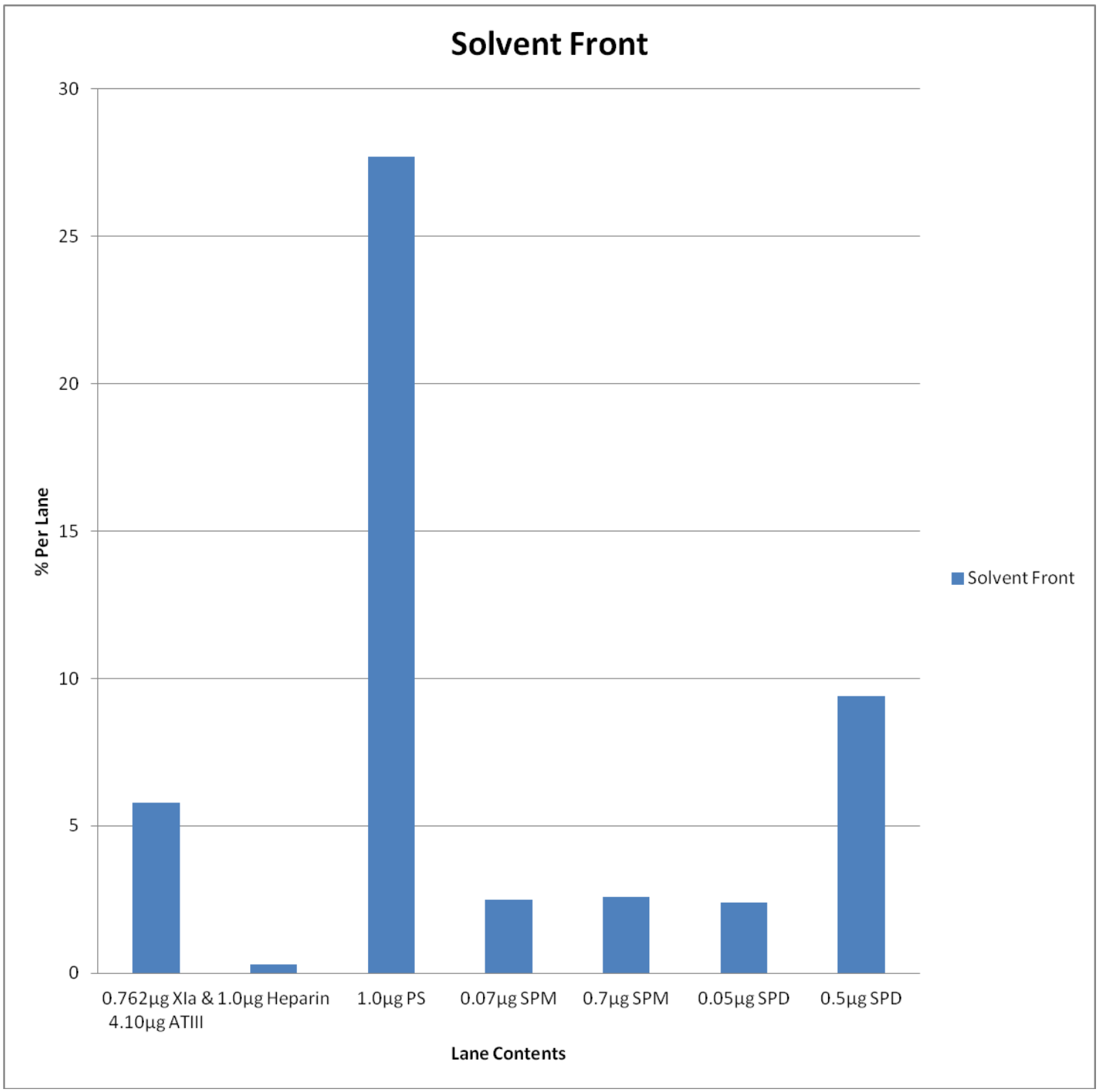


Table 17: Solvent Front Analysis Per Lane

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Solvent Front	5.8	0.3	27.7	2.5	2.6	2.4	9.4

Figure V: Graphical Representation of Table 17



Gel 4

Table 18: Overall Protein Analysis Per Lane

	0.762µg Xla & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
[Xla-$(ATIII)_2$]	0.7	5.1	*ND	*ND	*ND	*ND	*ND
[Xla-ATIII]	12.1	18.7	5.1	5.6	3.3	2	1.6
Xla	28.8	11.2	32.9	36.8	39.4	60.6	76.6
ATIII	49.9	64	39.5	53.8	52.5	30.8	10.1
Solvent Front	8.4	1.3	22.5	3.8	4.8	6.6	11.7

*ND= Not Detected

Standard Deviation	22.26996741	Mean	23.34	Standard Error	3.764312

Figure W: Graphical Representation of Table 18

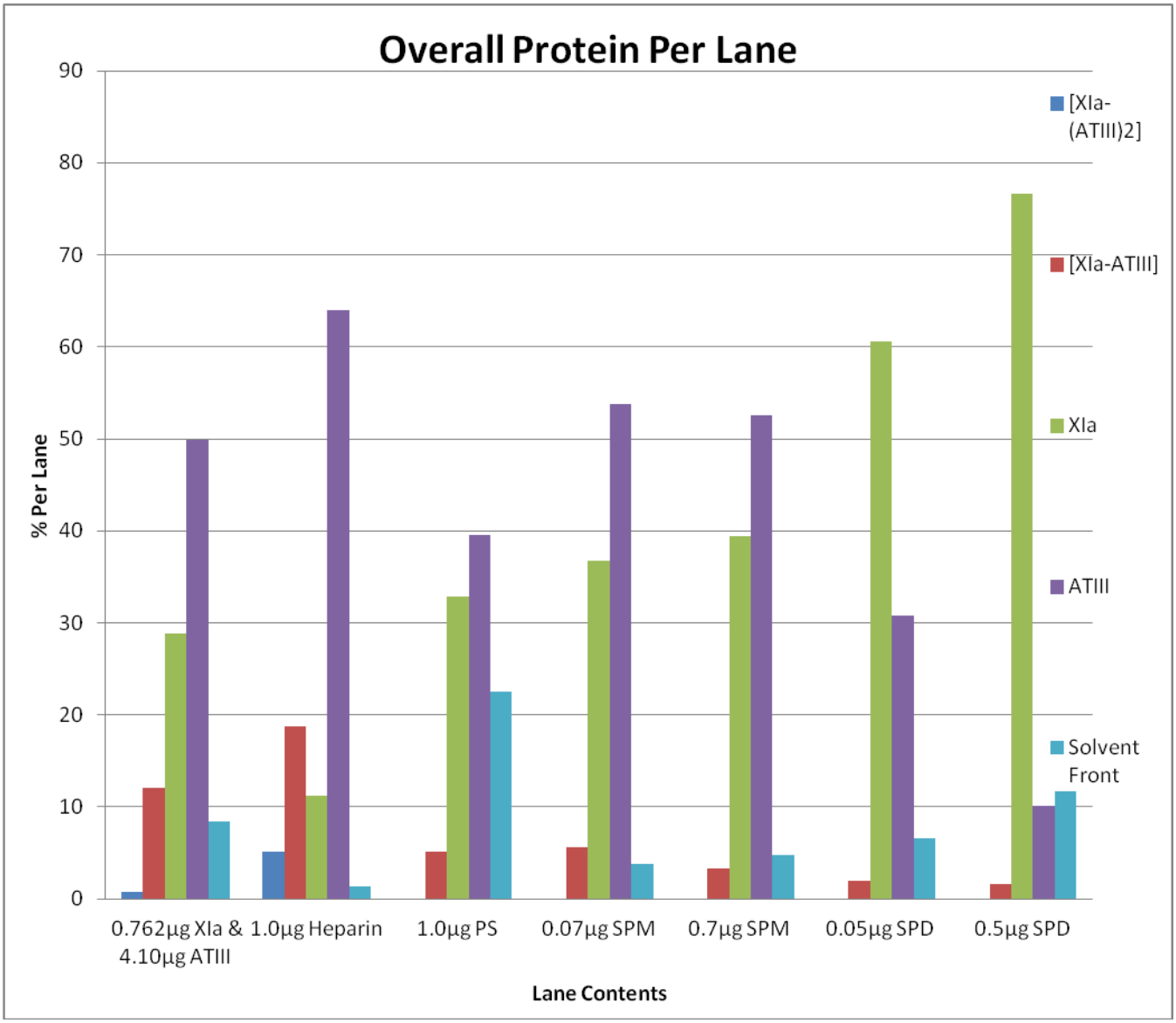


Table 19: [XIa-$(ATIII)_2$] Analysis Per Lane

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
[XIa-$(ATIII)_2$]	0.7	5.1	*ND	*ND	*ND	*ND	*ND

*ND= Not Detected

Figure X: Graphical Representation of Table 19

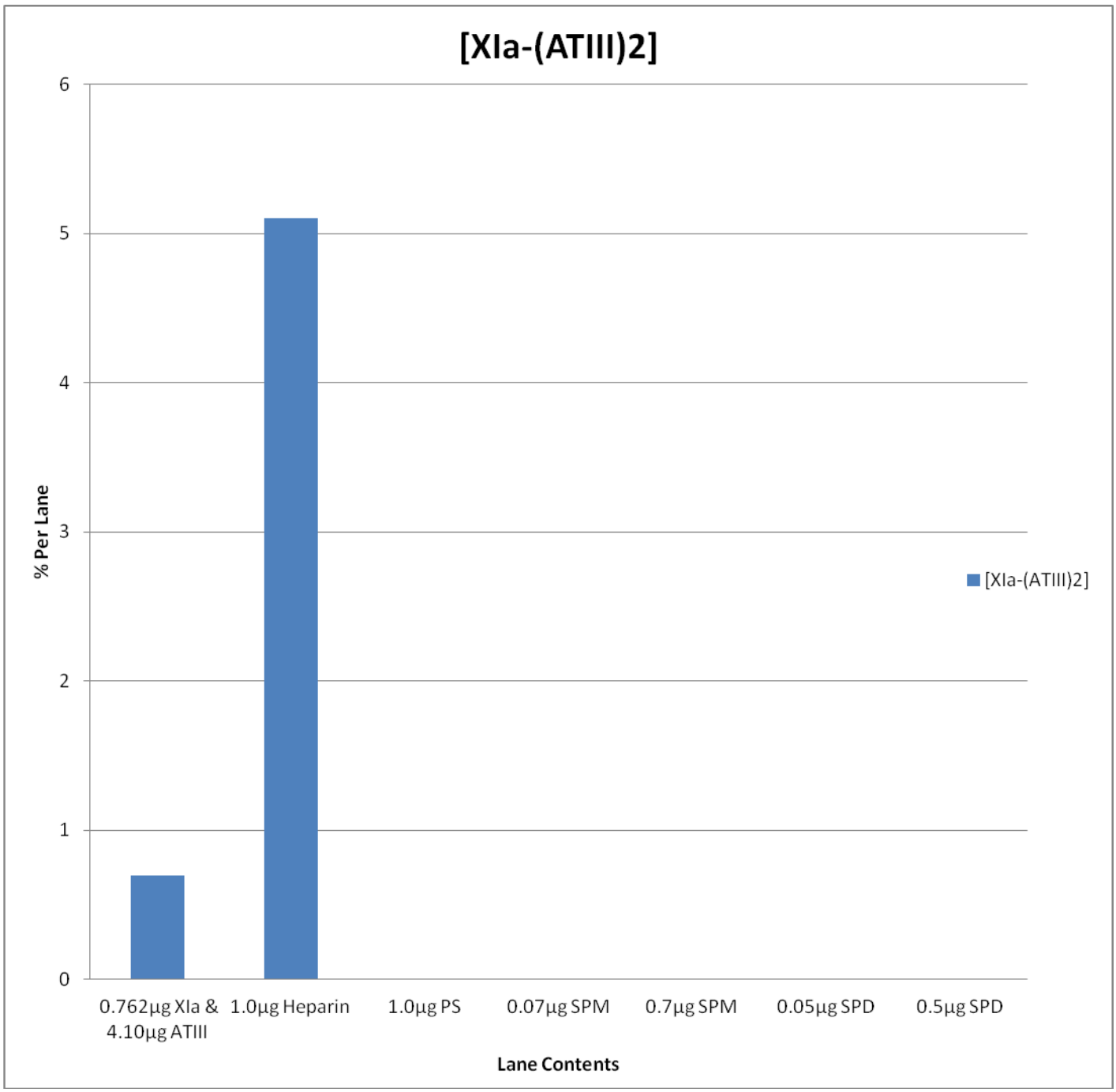


Table 20: [XIa-ATIII] Analysis Per Lane

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
[XIa-ATIII]	12.1	18.7	5.1	5.6	3.3	2	1.6

Figure Y: Graphical Representation of Table 20

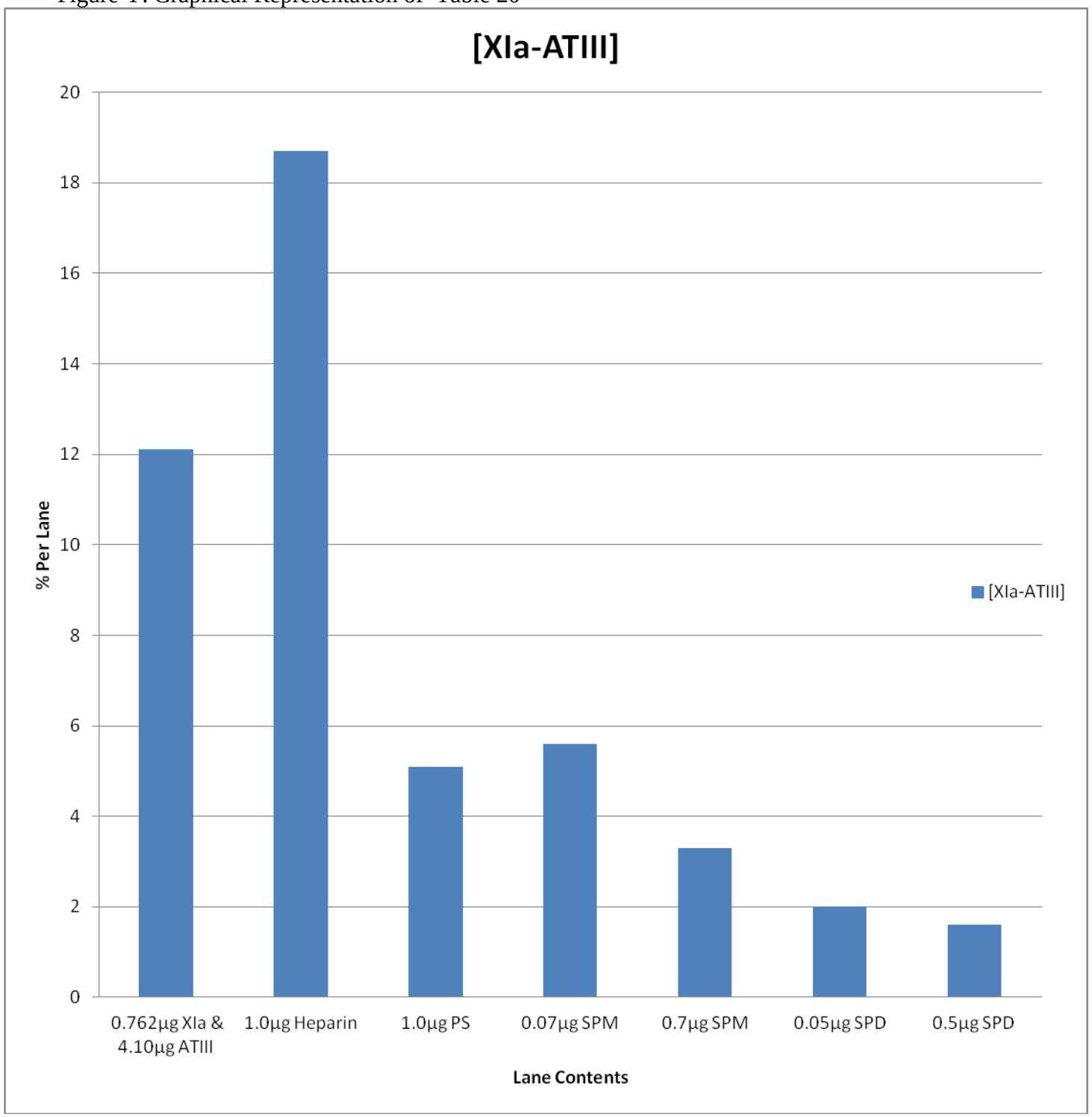


Table 21: XIa Analysis Per Lane

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
XIa	28.8	11.2	32.9	36.8	39.4	60.6	76.6

Figure Z: Graphical Representation of Table 21

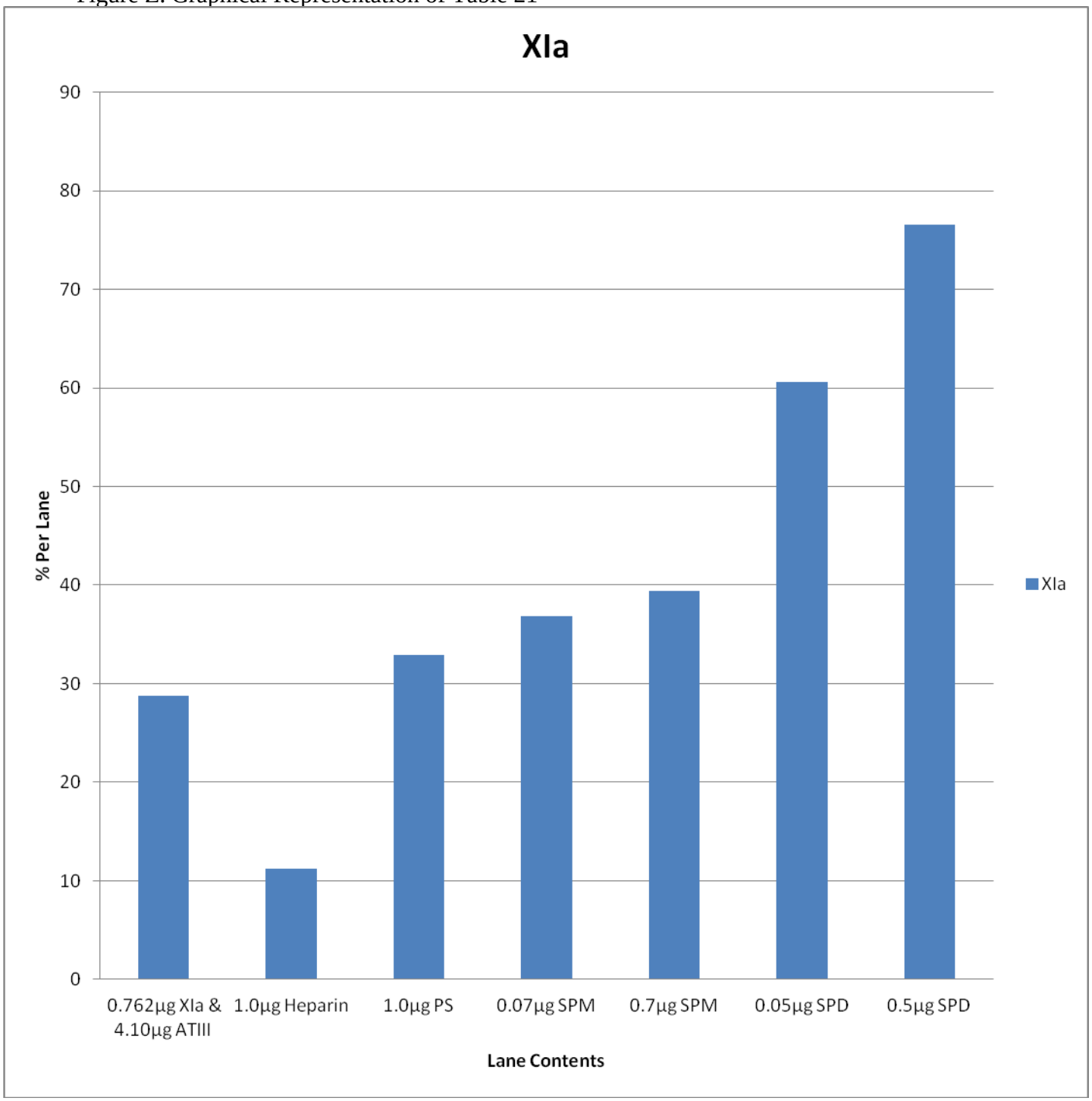


Table 22: ATIII Analysis Per Lane

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIII	49.9	64	39.5	53.8	52.5	30.8	10.1

Figure AA: Graphical Representation of Table 22

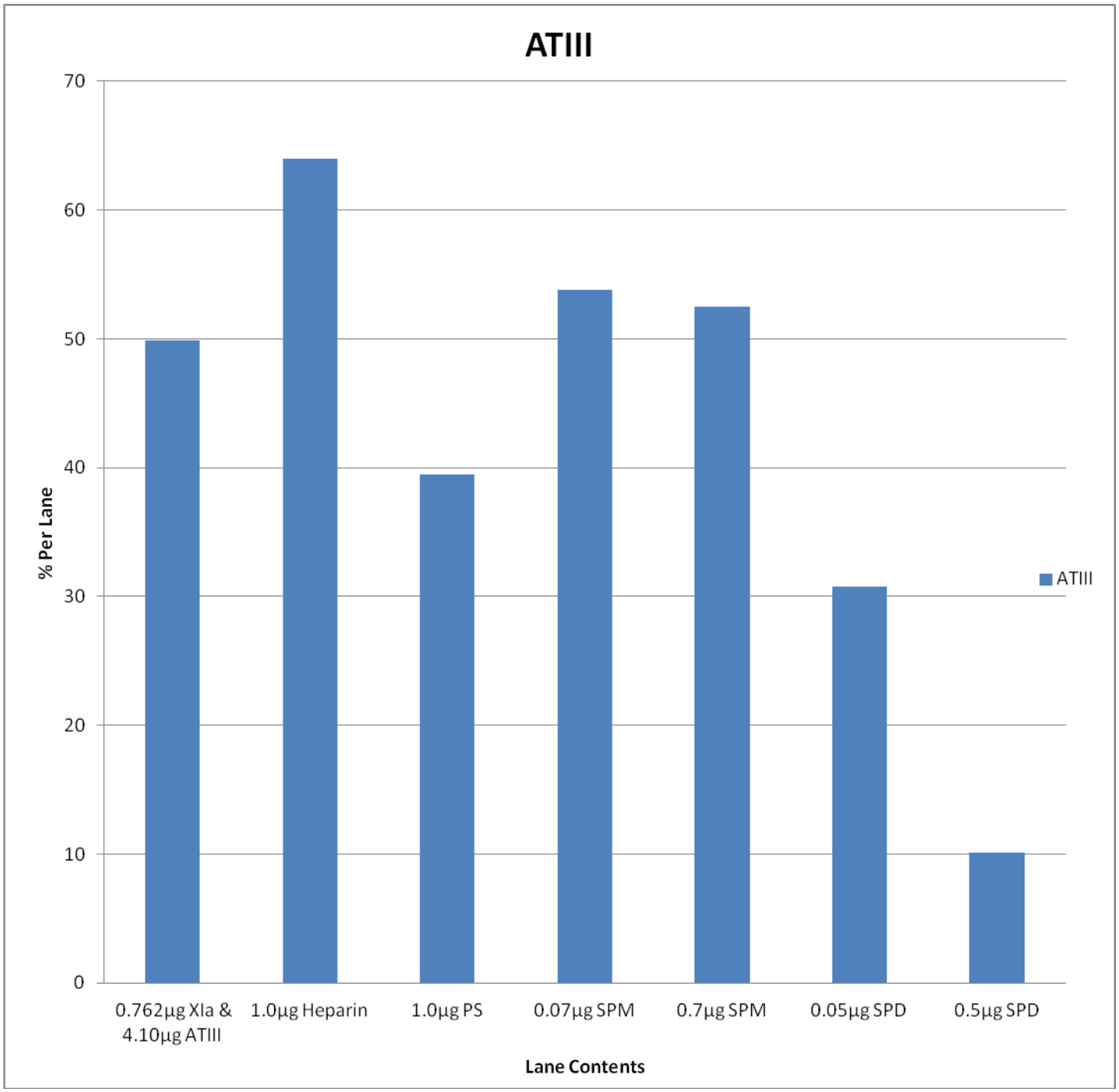
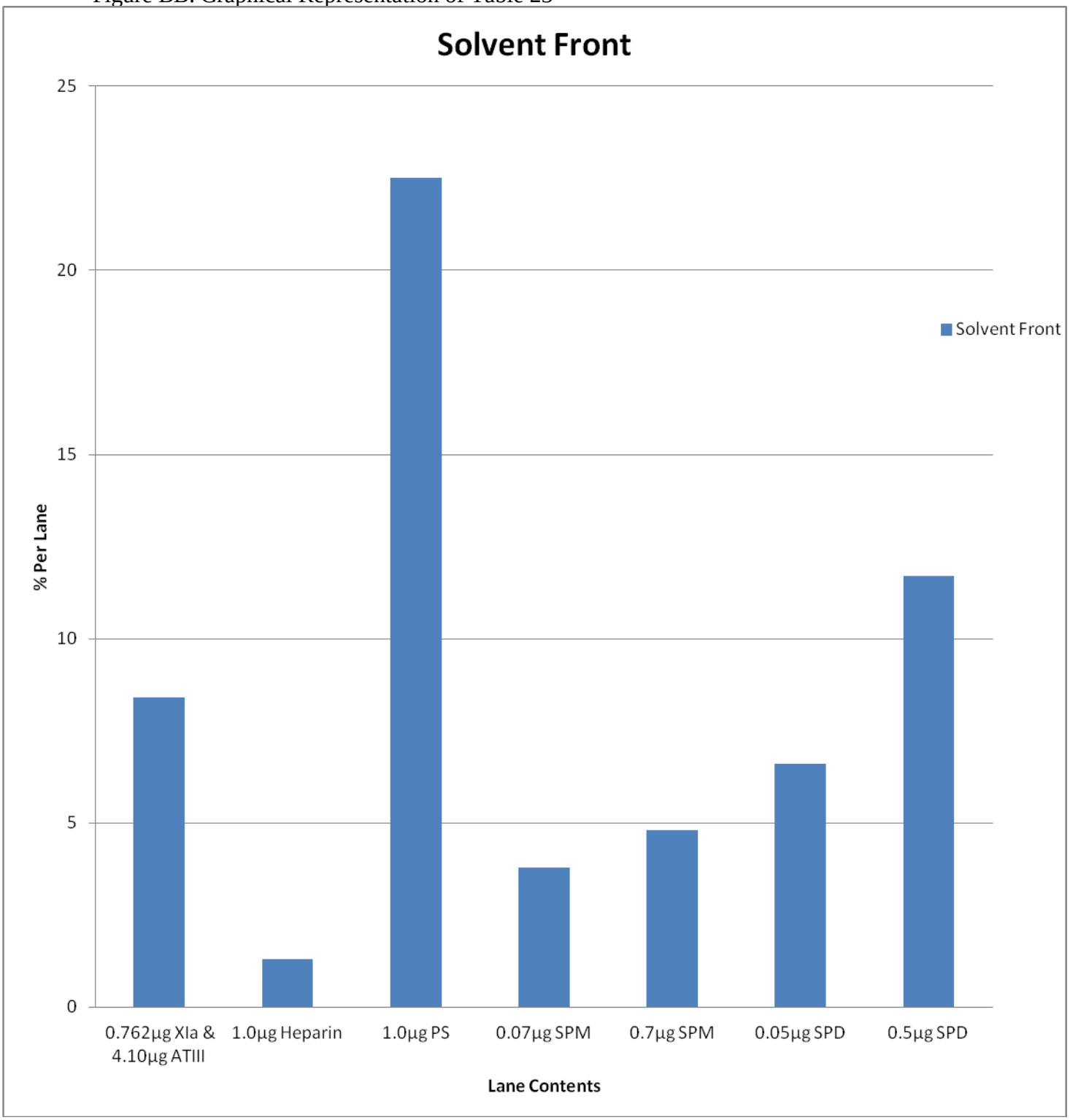


Table 23: Solvent Front Analysis Per Lane

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Solvent Front	8.4	1.3	22.5	3.8	4.8	6.6	11.7

Figure BB: Graphical Representation of Table 23



P Value Tables:
Tables created and used for analysis

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
[XIa-(ATIII)2]	0.9	2.4	*ND	*ND	*ND	*ND	*ND
[XIa-(ATIII)2]	0.4	1.7	*ND	*ND	*ND	*ND	*ND
[XIa-(ATIII)2]	0.6	4.8	*ND	*ND	*ND	*ND	*ND
[XIa-(ATIII)2]	0.7	5.1	*ND	*ND	*ND	*ND	*ND

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
[XIa-ATIII]	12.1	19.5	7.1	8.1	8.7	7.2	5
[XIa-ATIII]	11.6	19.4	6.7	7.6	7.5	6.6	4.2
[XIa-ATIII]	11	16.6	4.1	4.8	4.4	1.7	1.6
[XIa-ATIII]	12.1	18.7	5.1	5.6	3.3	2	1.6

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
XIa	29.9	31.9	35.2	41.5	40	40	25.2
XIa	30.3	33.9	35.5	42.1	40.6	40.6	27.3
XIa	28.4	10.8	31.2	32.8	41.1	66.9	81.1
XIa	28.8	11.2	32.9	36.8	39.4	60.6	76.6

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIII	52.8	45.4	43.6	47.4	47.6	49.5	67.2
ATIII	53.5	44.1	45.1	47	48.6	50.3	65.9
ATIII	54.3	67.4	37	59.9	51.8	28.9	7.9
ATIII	49.9	64	39.5	53.8	52.5	30.8	10.1

	0.762µg XIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Solvent Front	4.3	0.8	14	3.1	3.7	3.4	2.6
Solvent Front	4.2	0.9	12.6	3.3	3.4	2.5	2.6
Solvent Front	5.8	0.3	27.7	2.5	2.6	2.4	9.4
Solvent Front	8.4	1.3	22.5	3.8	4.8	6.6	11.7

P Values for [XIa-$(ATIII)_2$] Complex

[XIa-(ATIII)2]
P Values for [XIa-(ATIII)2]

	0.762µg XIa & 4.10µg ATIII	*1.0µg Heparin*
Mean	0.65	3.5
Variance	0.043333333	2.9
Observations	4	4
P(T<=t) one-tail	0.021288364	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.042576728	
t Critical two-tail	3.182446305	

	0.762µg XIa & 4.10µg ATIII	*1.0µg PS*
Mean	0.65	0
Variance	0.043333333	0
t Stat	6.244997998	
P(T<=t) one-tail	0.004141335	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.008282669	
t Critical two-tail	3.182446305	

	0.762µg XIa & 4.10µg ATIII	*0.07µg SPM*
Mean	0.65	0
Variance	0.043333333	0
t Stat	6.244997998	
P(T<=t) one-tail	0.004141335	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.008282669	
t Critical two-tail	3.182446305	

	0.762µg XIa & 4.10µg ATIII	*0.7µg SPM*
Mean	0.65	0
Variance	0.043333333	0
t Stat	6.244997998	
P(T<=t) one-tail	0.004141335	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.008282669	
t Critical two-tail	3.182446305	

	0.762µg XIa & 4.10µg ATIII	*0.05 SPDµg PS*
Mean	0.65	0
Variance	0.043333333	0
t Stat	6.244997998	

P(T<=t) one-tail	0.004141335
t Critical one-tail	2.353363435
P(T<=t) two-tail	0.008282669
t Critical two-tail	3.182446305

	0.762µg XIa & 4.10µg ATIII	*0.5µg SPD*
Mean	0.65	0
Variance	0.043333333	0
t Stat	6.244997998	
P(T<=t) one-tail	0.004141335	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.008282669	
t Critical two-tail	3.182446305	

P Values for [XIa-ATIII]

[XIa-ATIII]

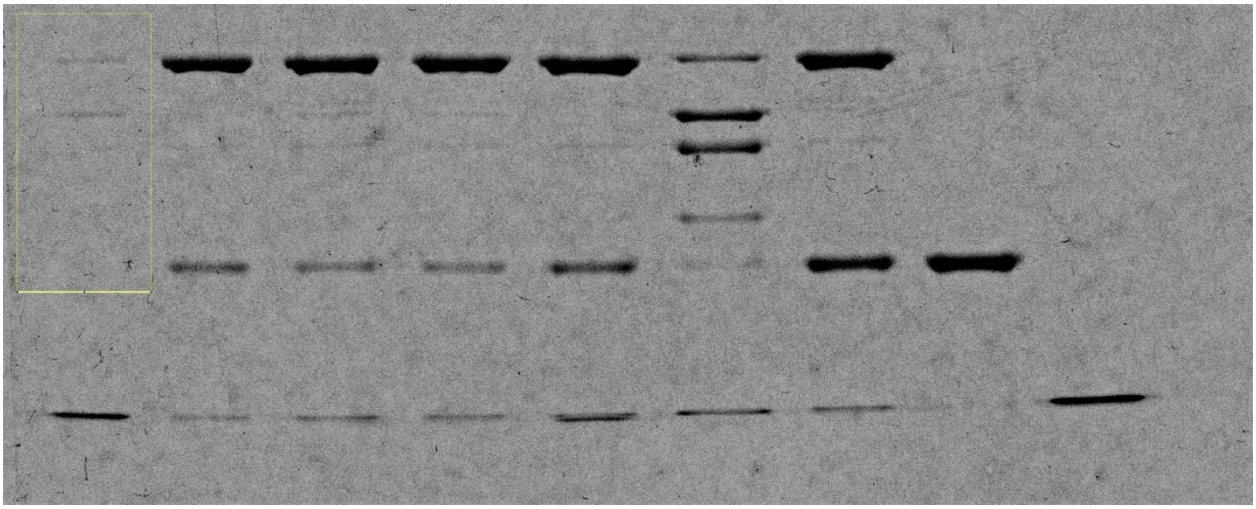
	0.762µg XIa & 4.10µg ATIII	*1.0µg Heparin*
Mean	11.7	18.55
Variance	0.273333333	1.816666667
t Stat	-14.10548348	
P(T<=t) one-tail	0.000385899	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.000771799	
t Critical two-tail	3.182446305	
	0.762µg XIa & 4.10µg ATIII	*1.0µg PS*
Mean	11.7	5.75
Variance	0.273333333	1.956666667
t Stat	10.29284428	
P(T<=t) one-tail	0.000977846	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.001955692	
t Critical two-tail	3.182446305	
	0.762µg XIa & 4.10µg ATIII	*0.07µg SPM*
Mean	11.7	6.525
Variance	0.273333333	2.489166667
t Stat	7.597502721	
P(T<=t) one-tail	0.002365834	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.004731668	
t Critical two-tail	3.182446305	
	0.762µg XIa & 4.10µg ATIII	*0.7µg SPM*
Mean	11.7	5.975
Variance	0.273333333	6.4625
t Stat	4.640091621	
P(T<=t) one-tail	0.009432442	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.018864884	
t Critical two-tail	3.182446305	
	0.762µg XIa & 4.10µg ATIII	*0.05µg SPD*
Mean	11.7	4.375
Variance	0.273333333	8.575833333
t Stat	5.303945751	
P(T<=t) one-tail	0.006541575	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.01308315	
t Critical two-tail	3.182446305	
	0.762µg XIa & 4.10µg ATIII	*0.5µg SPD*

Mean	11.7	3.1
Variance	0.273333333	3.106666667
t Stat	10.57252749	
P(T<=t) one-tail	0.000903839	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.001807678	
t Critical two-tail	3.182446305	

Brief Analysis:
[XIa-(ATIII)2] and [XIa-ATIII] are formed upon preincubating XIa and ATIII at room temperature for thirty minutes. Corresponding to 0.4% of total protein per lane, this is increased in the presence of 3ug of Heparin.
However, upon addition of 3ug of Protamine Sulfate, 0.07ug of Spermine, 0.7ug Spermine, 0.05ug Spermidine, and 0.5ug Spermidine, to the Xia prior to the addition of ATIII prevented or inhibited the formation of the [XIa-ATIII] complex.
Furthermore, 10.7% of the protein in the lane is represented by a major degradation of [XIa-ATIII]. The band increases to 19.4% in the presence of Heparin and is decreased to 5.9% with Protamine Sulfate, 6.8% with 0.07ug Spermine, 7.2% with 0.7 Spermine, 6.1% with 0.05ug Spermidine, 4.3% with 0.5ug Spermine.

IIa

The Effects of Heparin, Protamine Sulfate, Sperimine, and Spermidine on the Interaction between Factor IIa and Anti-Thrombin III



1. 5µL Molecular Weight Markers+ 13µL Tris/NaCl pH8 + 5µL 5X Sample Buffer
2. 2.0µL (2.45µg) FIIa + 13µL Tris/NaCl pH8 + 5µL 5X Sample Buffer
3. 2.0µL (4.10µg) ATIII + 13µL Tris/NaCl pH8 + 5µL 5X Sample Buffer
4. 2.0µL (2.45µg) FIIa + 2.0µL (4.10µg) ATIII + 11µL Tris/NaCl pH8 + 5µL 5X Sample Buffer
5. [(2.0µL (4.10µg) ATIII + 1µg (3µL) Heparin) 15’ at RT, + 2.0µL (2.45µg) FIIa + 8µL Tris/NaCl pH8] 60’ at RT, + 5µL 5X Sample Buffer]
6. [(2.0µL (4.10µg) ATIII + 1µg (3µL) Protamine Sulfate) 15’ at RT, + 2.0µL (2.45µg) FIIa + 8µL Tris/NaCl pH8] 60’ at RT, + 5µL 5X Sample Buffer]
7. [(2.0µL (4.10µg) ATIII + 0.07µg (3µL) Spermine) 15’ at RT, + 2.0µL (2.45µg) FIIa + 8µL Tris/NaCl pH8] 60’ at RT, + 5µL 5X Sample Buffer]
8. [(2.0µL (4.10µg) ATIII + 0.7µg (3µL) Spermine) 15’ at RT, + 2.0µL (2.45µg) FIIa + 8µL Tris/NaCl pH8] 60’ at RT, + 5µL 5X Sample Buffer]
9. [(2.0µL (4.10µg) ATIII + 0.05µg (3µL) Spermidine) 15’ at RT, + 2.0µL (2.45µg) FIIa + 8µL Tris/NaCl pH8] 60’ at RT, + 5µL 5X Sample Buffer]
10. [(2.0µL (4.10µg) ATIII + 0.5µg (3µL) Spermidine) 15’ at RT, + 2.0µL (2.45µg) FIIa + 8µL Tris/NaCl pH8] 60’ at RT, + 5µL 5X Sample Buffer]

*Gels 1 and 2 were thrown out for inaccuracy
Pictures of Each Gel:
Gel 3 and 4

Figure A: Gel 3

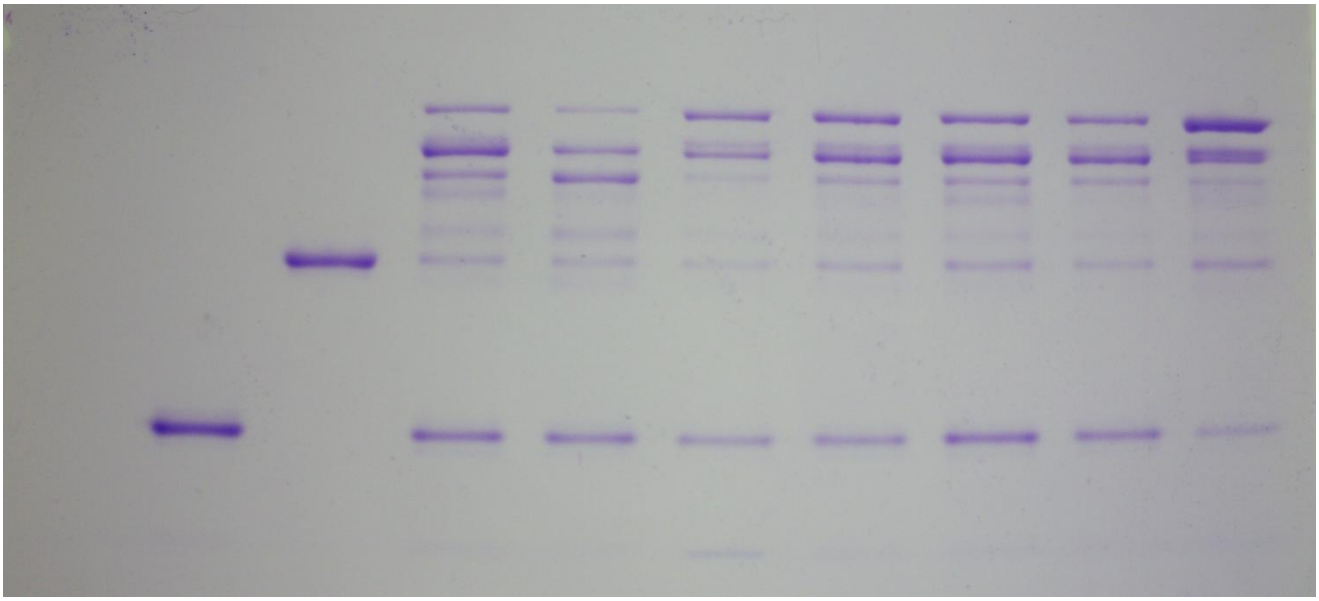


Figure B: Gel 4

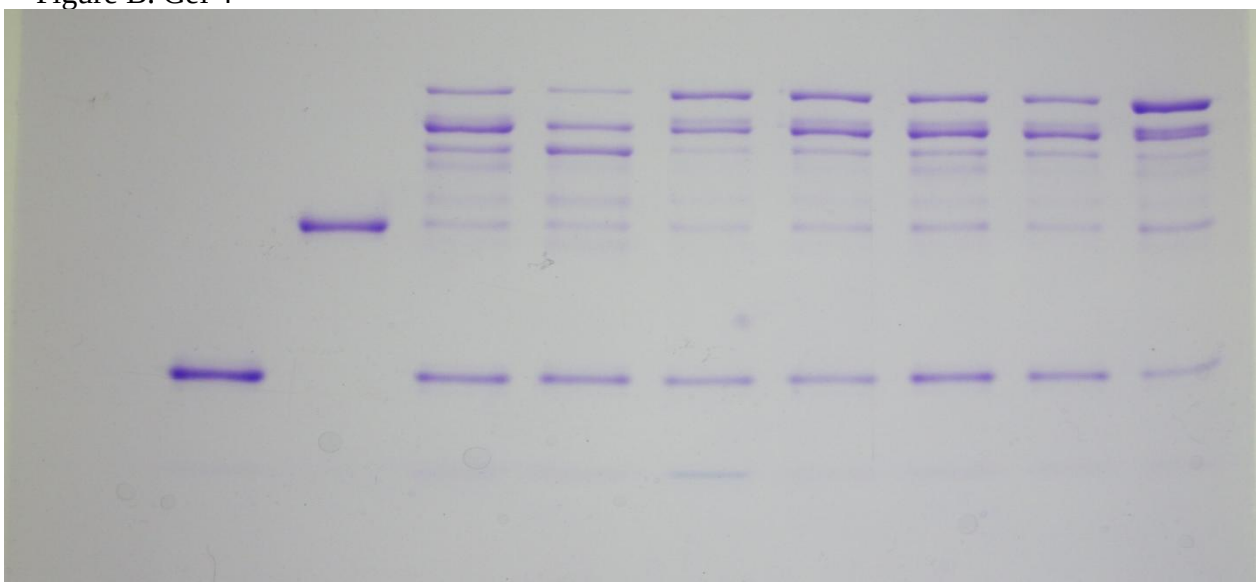


Table 1: Overall Protein Analysis Per Lane
*ND= Not Detected

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Alpha [IIa-ATIII]	13.8	6.4	37.7	34.2	24.7	24.8	51.9
Beta [IIa-ATIIII]	36.7	21.7	30.3	39.1	36.4	42.1	32.1
Gamma [IIa-ATIII]	12.3	34.2	4.24	6.9	9.36	8.27	2.59
ATIIIM	8.41	4.48	1.31	0.64	0.96	0.23	0.92
ATIII	5.76	5.74	3.69	4.74	5.85	3.93	7.43
IIa	23	27.5	19.2	14.4	22.8	20.7	5.04
Solvent Front	*ND	*ND	3.64	*ND	*ND	*ND	*ND

Standard Deviation	14.17008411	Mean	16.282093	Standard Error	2.024298

Figure C: Graphical Representation of Table 1

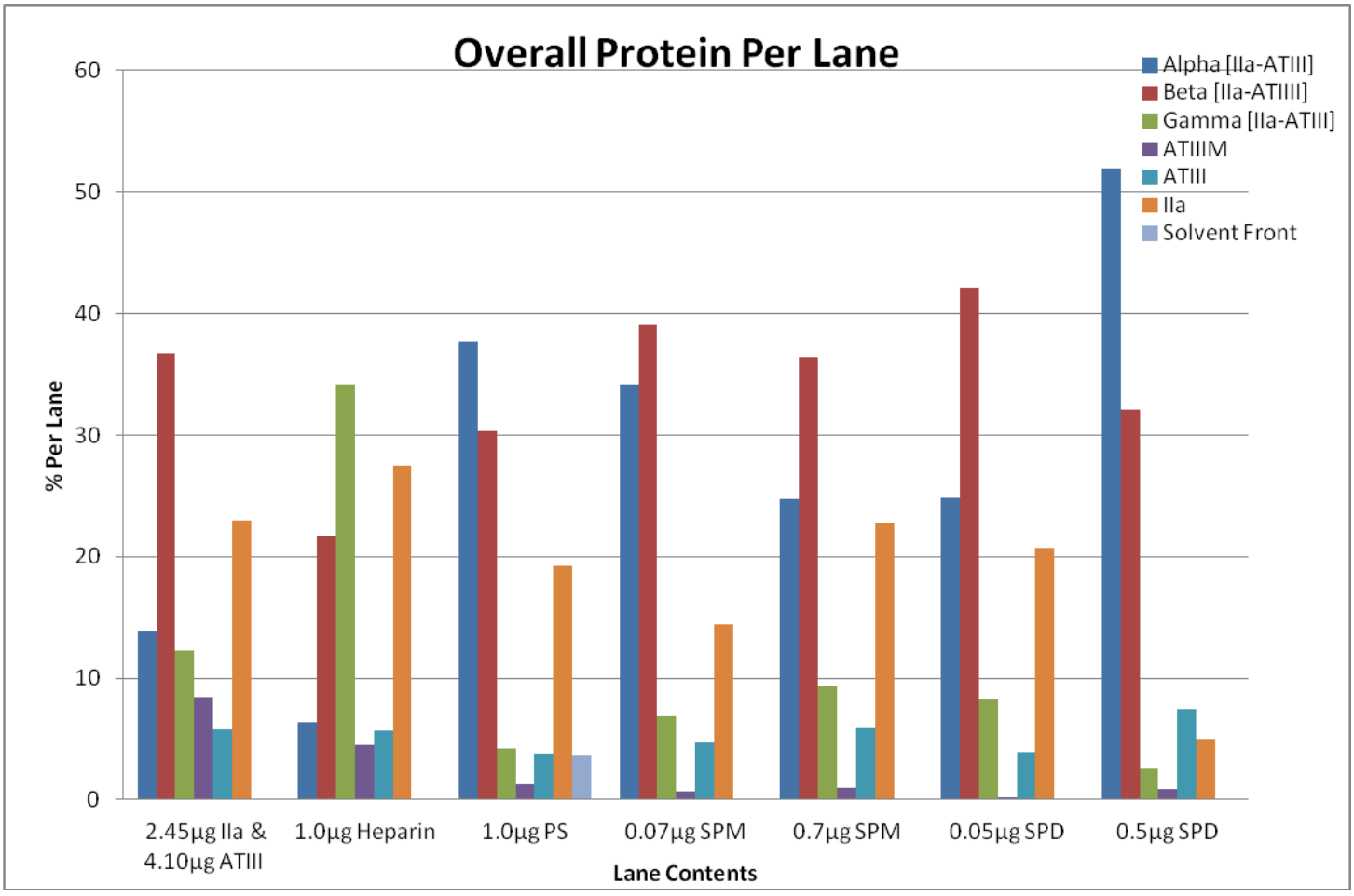


Table 2: Alpha [IIa-ATIII] Complex Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Alpha [IIa-ATIII]	13.8	6.4	37.7	34.2	24.7	24.8	51.9

Figure D: Graphical Representation of Table 2

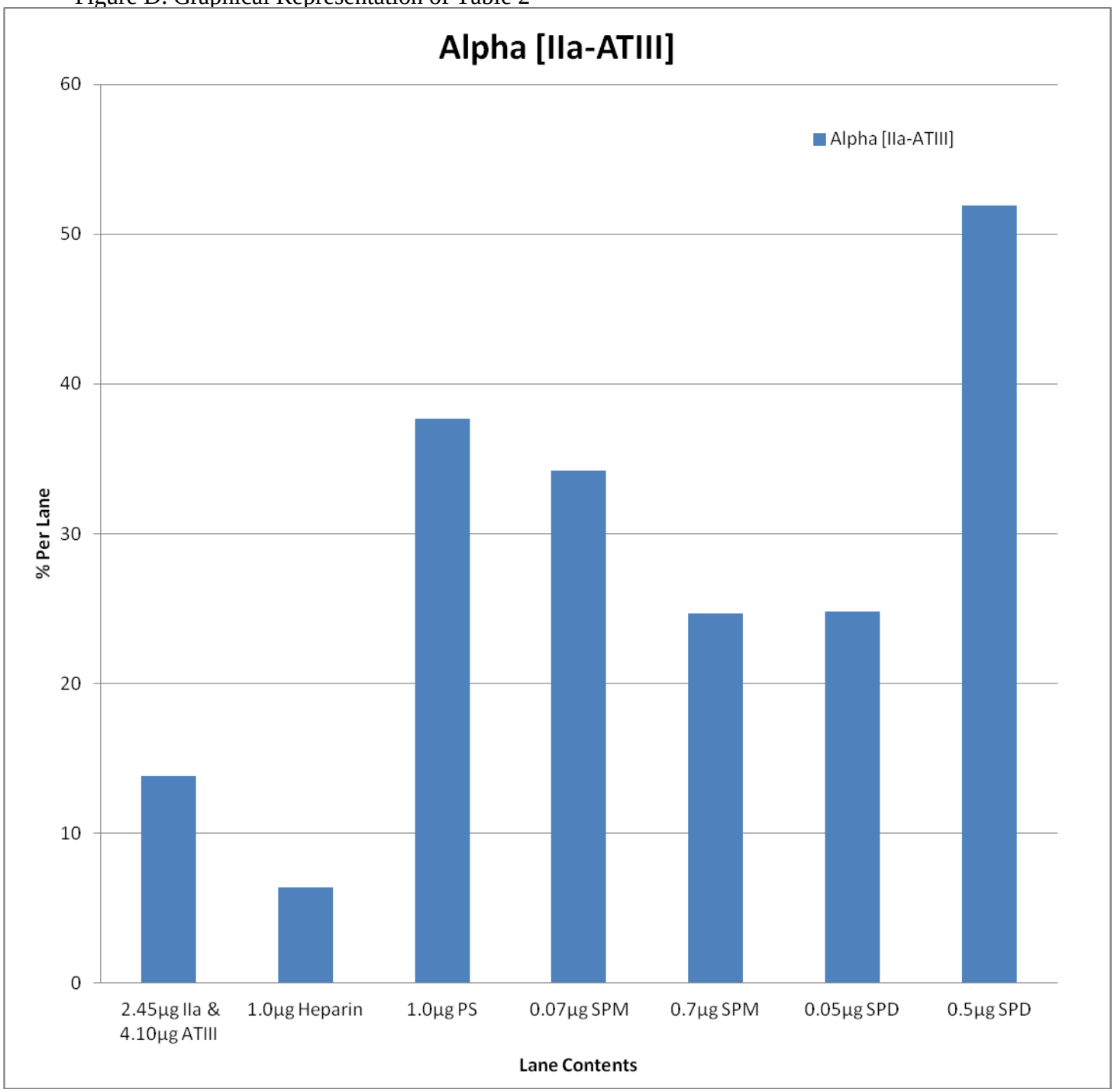


Table 3: Beta [IIa-ATIII] Complex Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Beta [IIa-ATIIII]	36.7	21.7	30.3	39.1	36.4	42.1	32.1

Figure E: Graphical Representation of Table 3

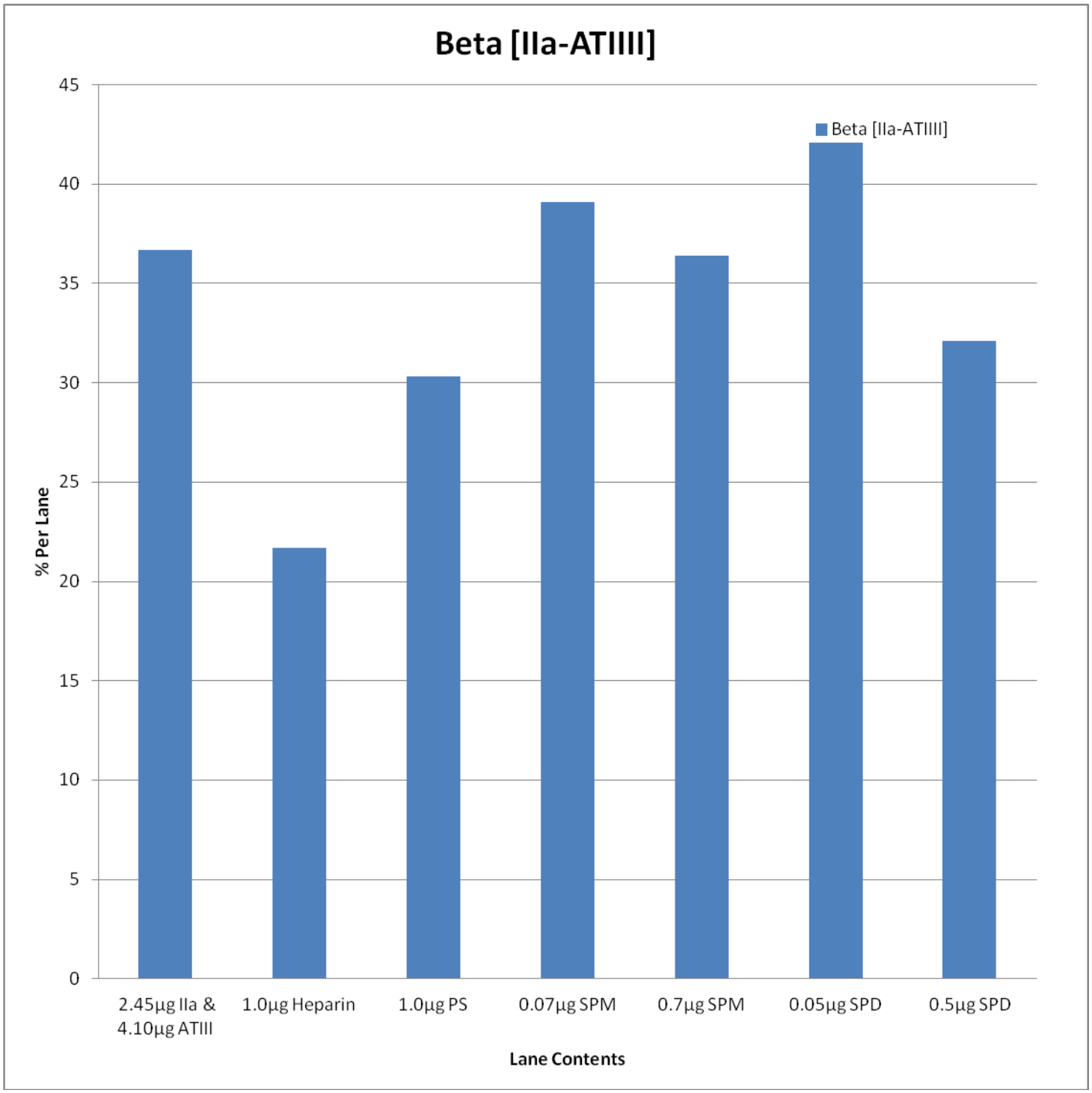


Table 4: Gamma [IIa-ATIII] Complex Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Gamma [IIa-ATIII]	12.3	34.2	4.24	6.9	9.36	8.27	2.59

Figure F: Graphical Representation of Table 4

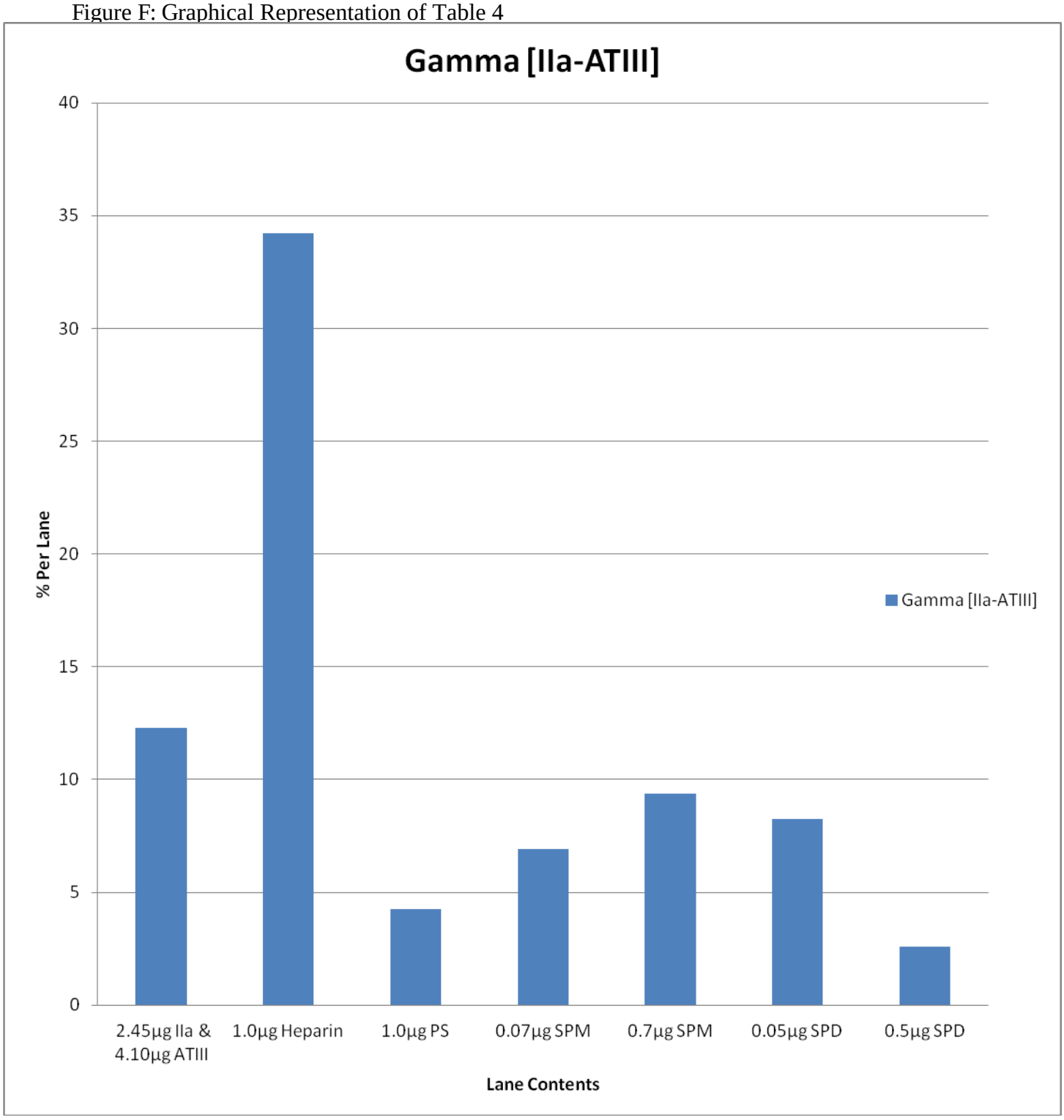


Table 5: ATIII-M Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIIIM	8.41	4.48	1.31	0.64	0.96	0.23	0.92

Figure G: Graphical Representation of Table 5

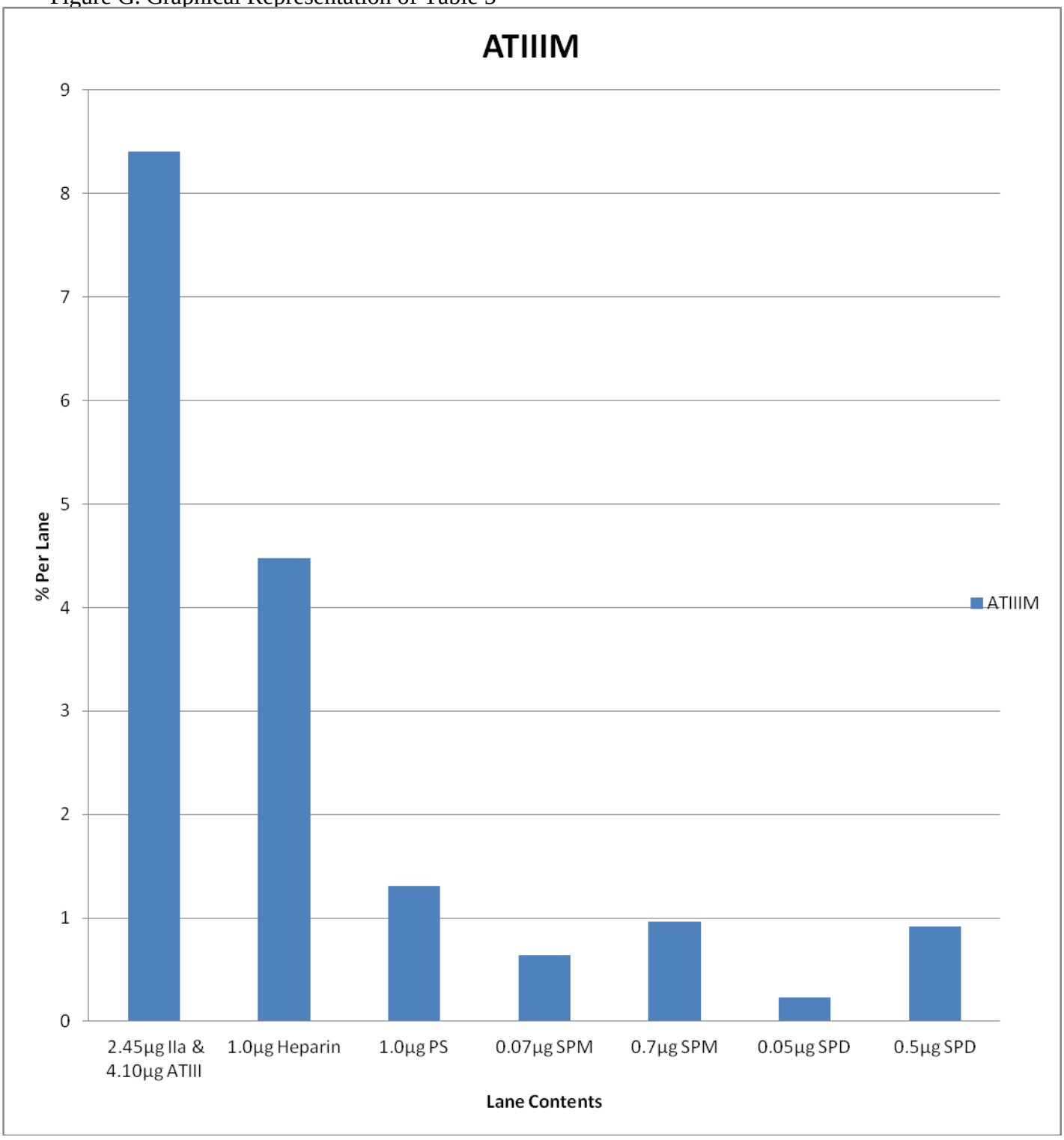


Table 6: ATIII Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIII	5.76	5.74	3.69	4.74	5.85	3.93	7.43

Figure H: Graphical Representation of Table 6

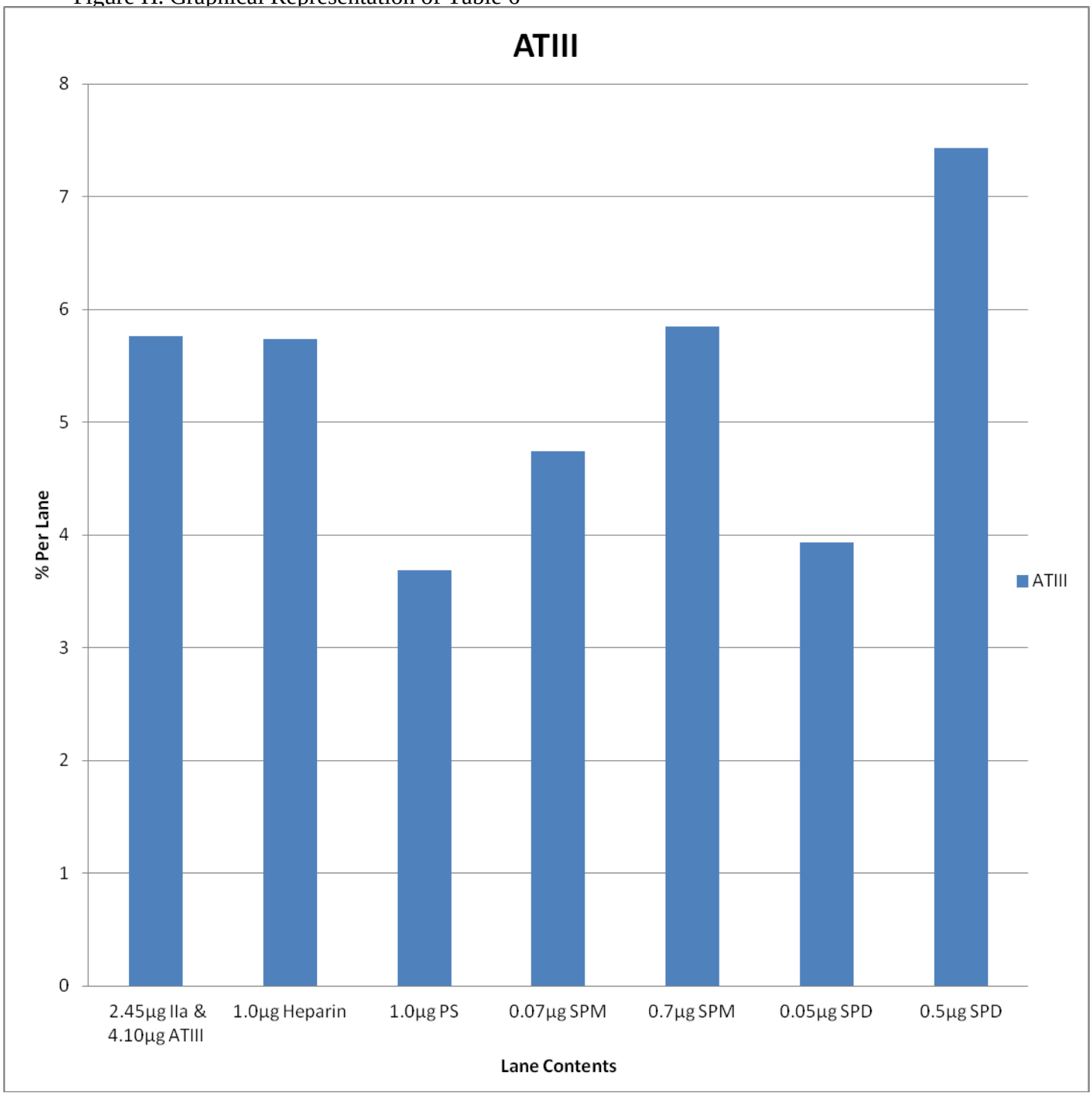


Table 7: IIa Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
IIa	23	27.5	19.2	14.4	22.8	20.7	5.04

Figure I: Graphical Representation of Table 7

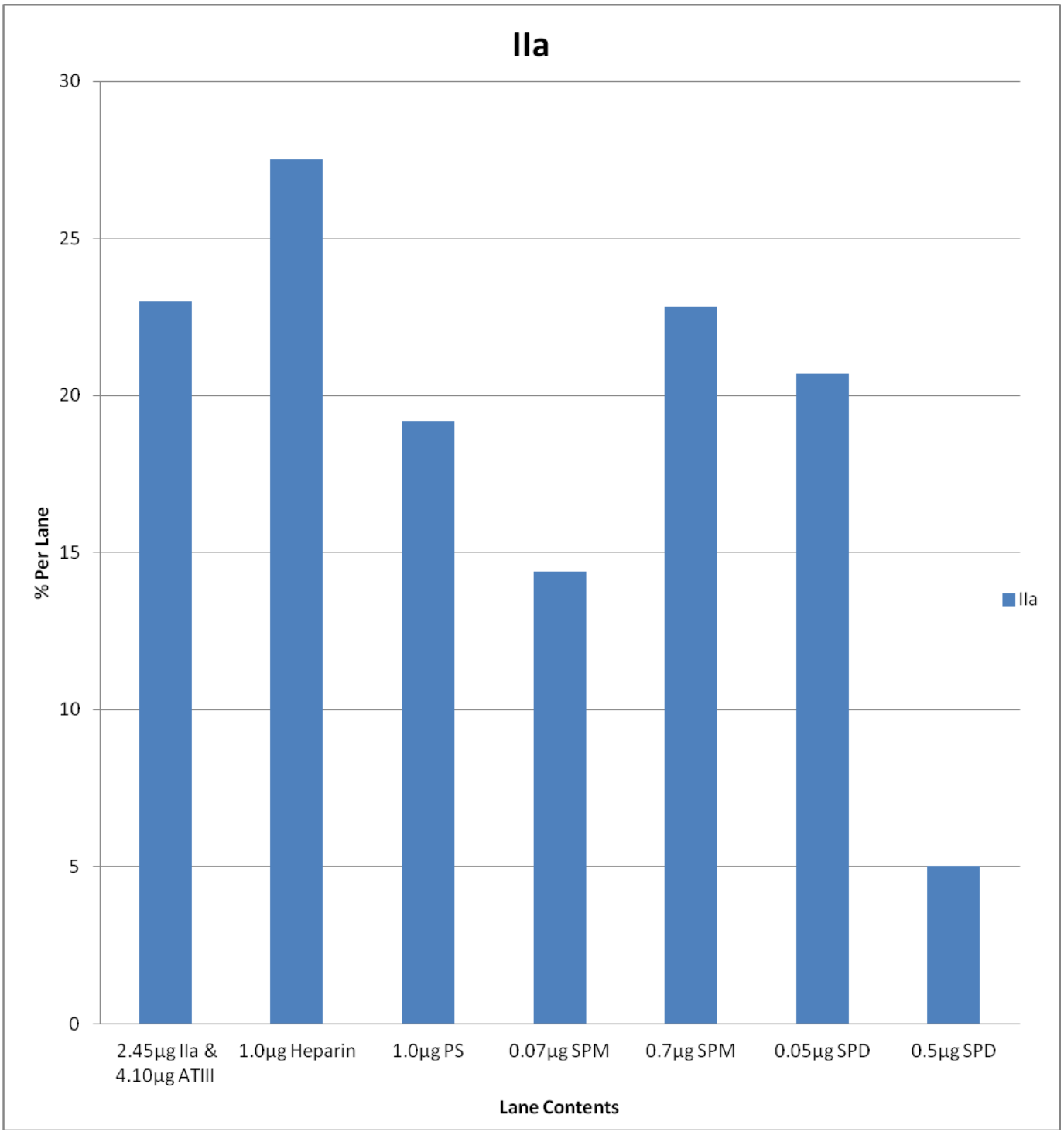
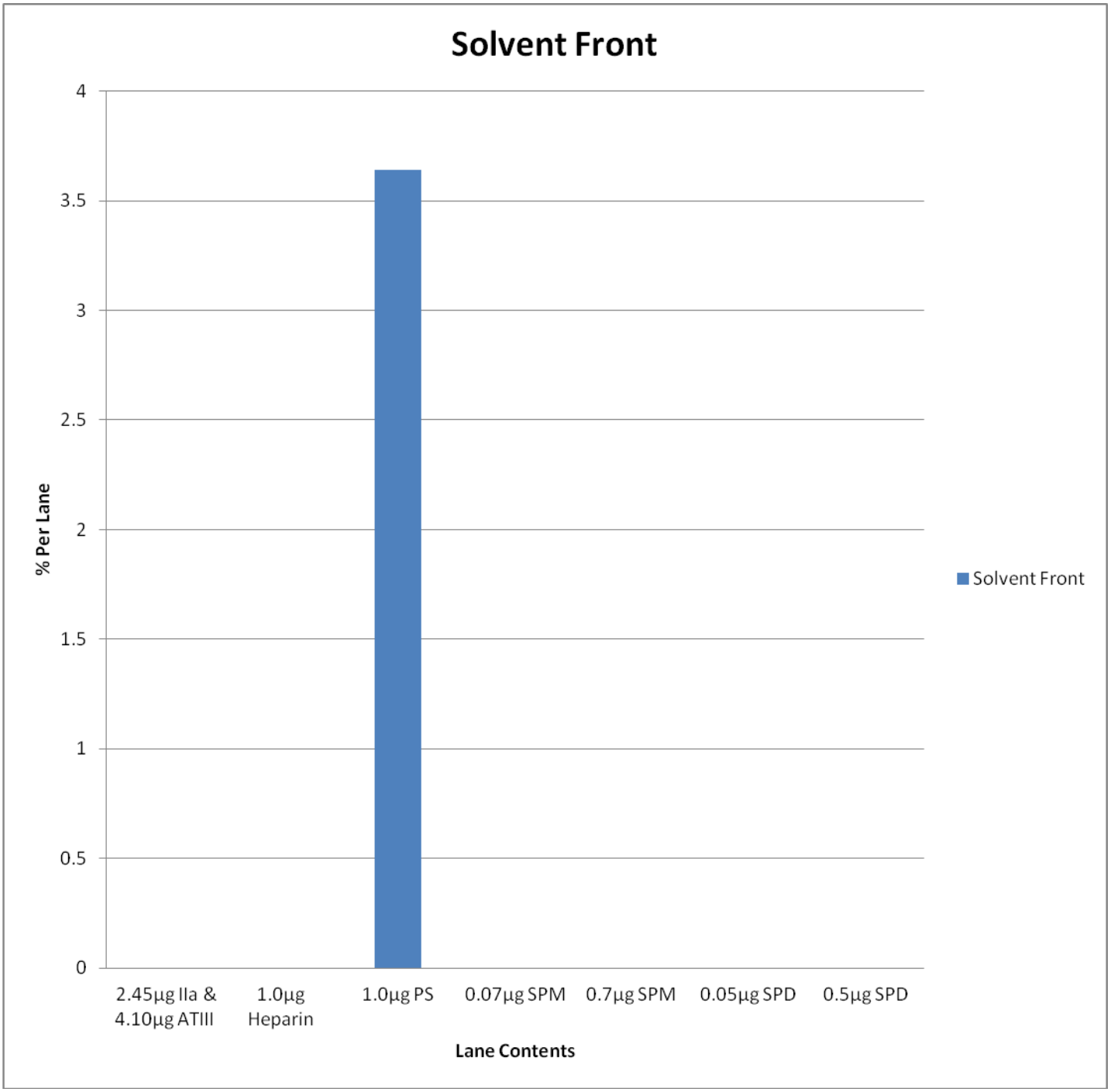


Table 8: Solvent Front Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Solvent Front	*ND	*ND	3.64	*ND	*ND	*ND	*ND

*ND= Not Detected

FigureJ: Graphical Representation of Table 8



Gel 4

Table 9: Overall Protein Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Alpha [IIa-ATIII]	13.7	5.88	35.7	32.9	24	23.2	54.1
Beta [IIa-ATIIII]	42.1	21	30.6	39.8	39	41.5	30.9
Gamma [IIa-ATIII]	11.8	33	3.65	6.92	6.21	7.75	2
ATIIIM	2.82	4.47	0.8	0.54	2.04	0.44	0.49
ATIII	3.73	5.16	2.8	4.12	5.08	3.75	5.93
IIa	25.9	30.5	20.5	15.7	23.7	23.4	6.52
Solvent Front	*ND	*ND	5.9	*ND	*ND	*ND	*ND

Standard Deviation	14.78176834	Mean	16.2790698	Standard Error	2.111681

*ND= Not Detected

Figure K: Graphical Representation of Table 9

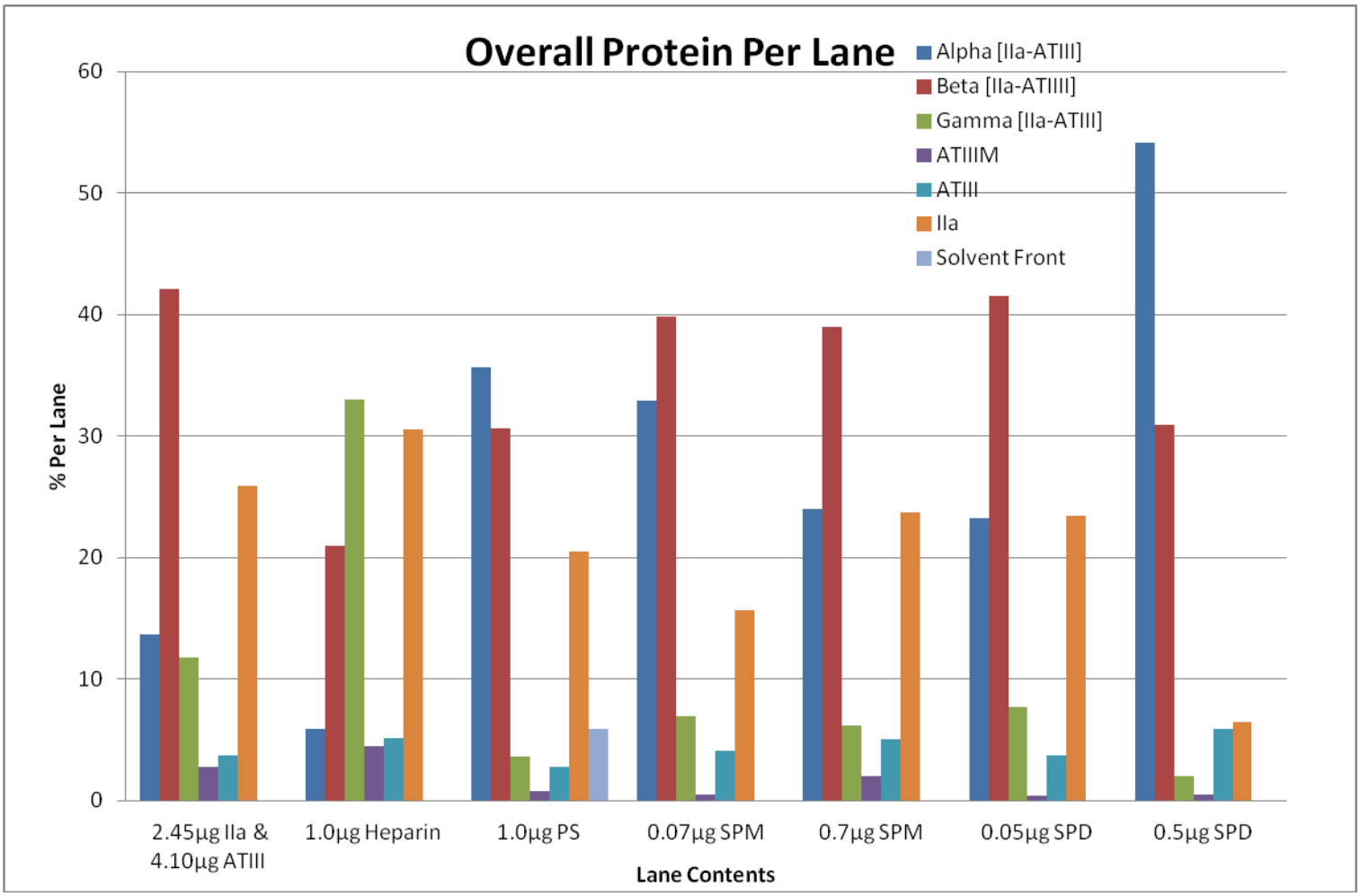


Table 10: Alpha [IIa-ATIII] Complex Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Alpha [IIa-ATIII]	13.7	5.88	35.7	32.9	24	23.2	54.1

Figure L: Graphical Representation of Table 10

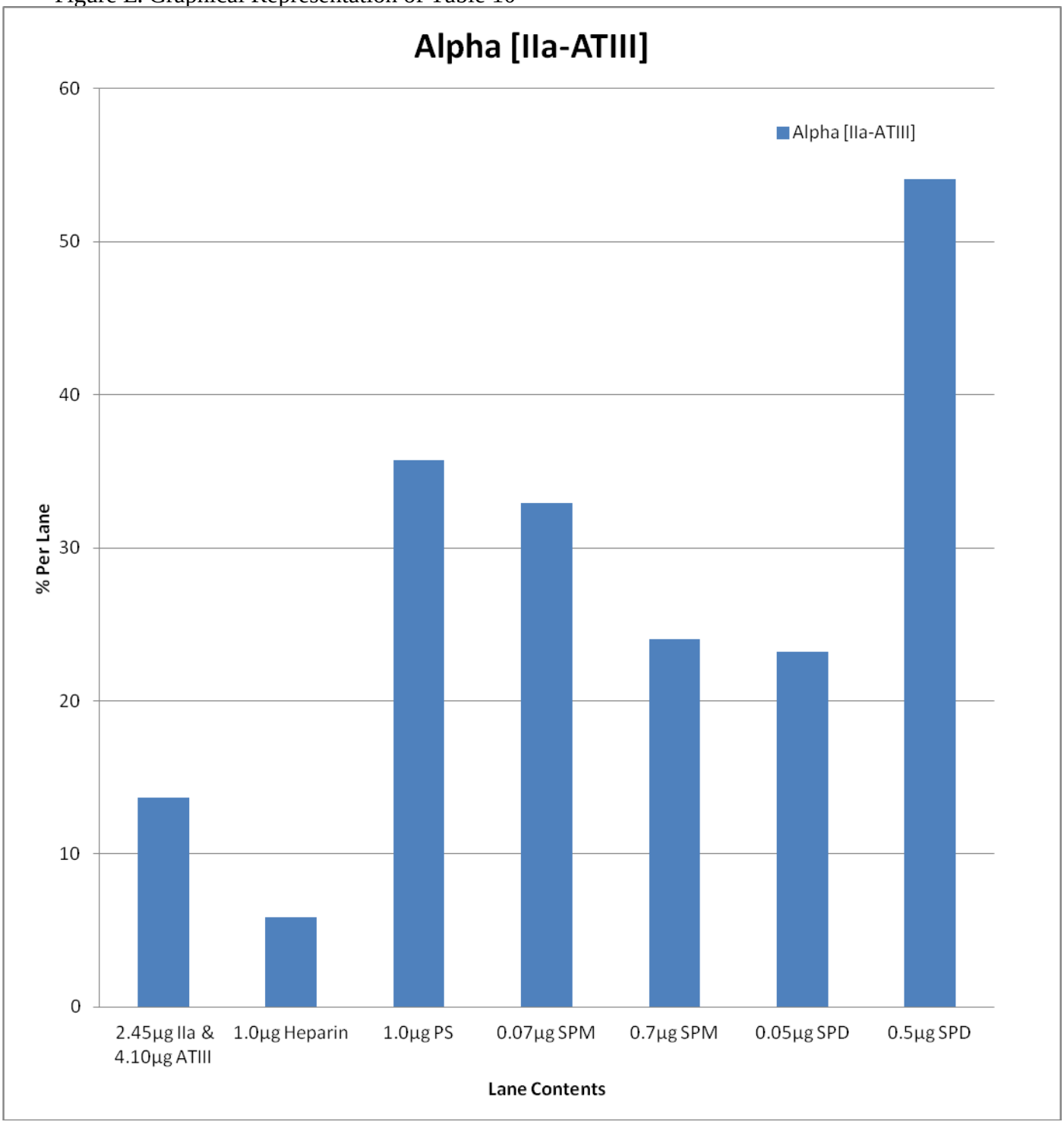


Table 11: Beta [IIa-ATIII] Complex Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Beta [IIa-ATIIII]	42.1	21	30.6	39.8	39	41.5	30.9

Figure M: Graphical Representation of Table 11

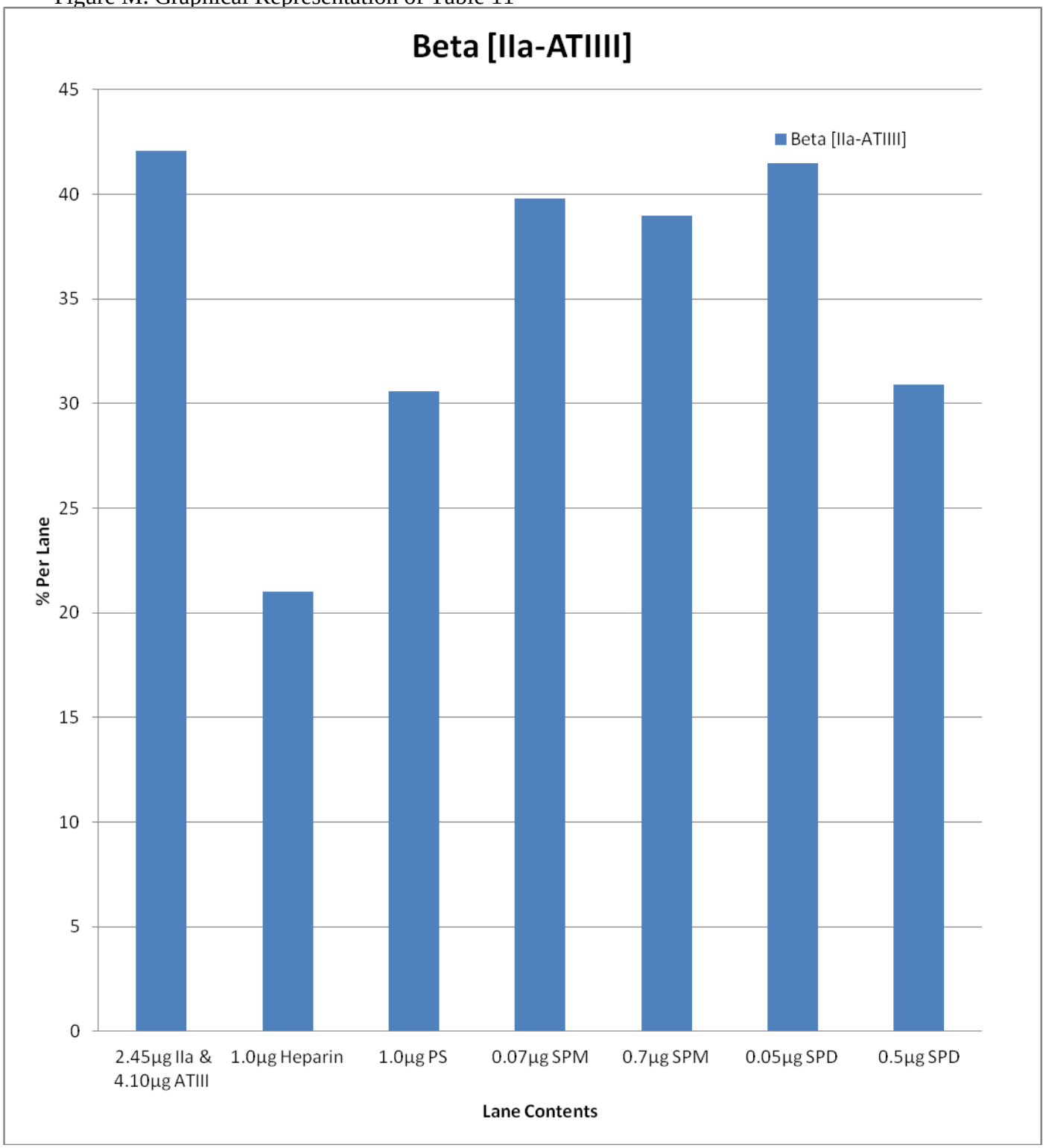


Table 12: Gamma [IIa-ATIII] Complex Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Gamma [IIa-ATIII]	11.8	33	3.65	6.92	6.21	7.75	2

Figure N: Graphical Representation of Table 12

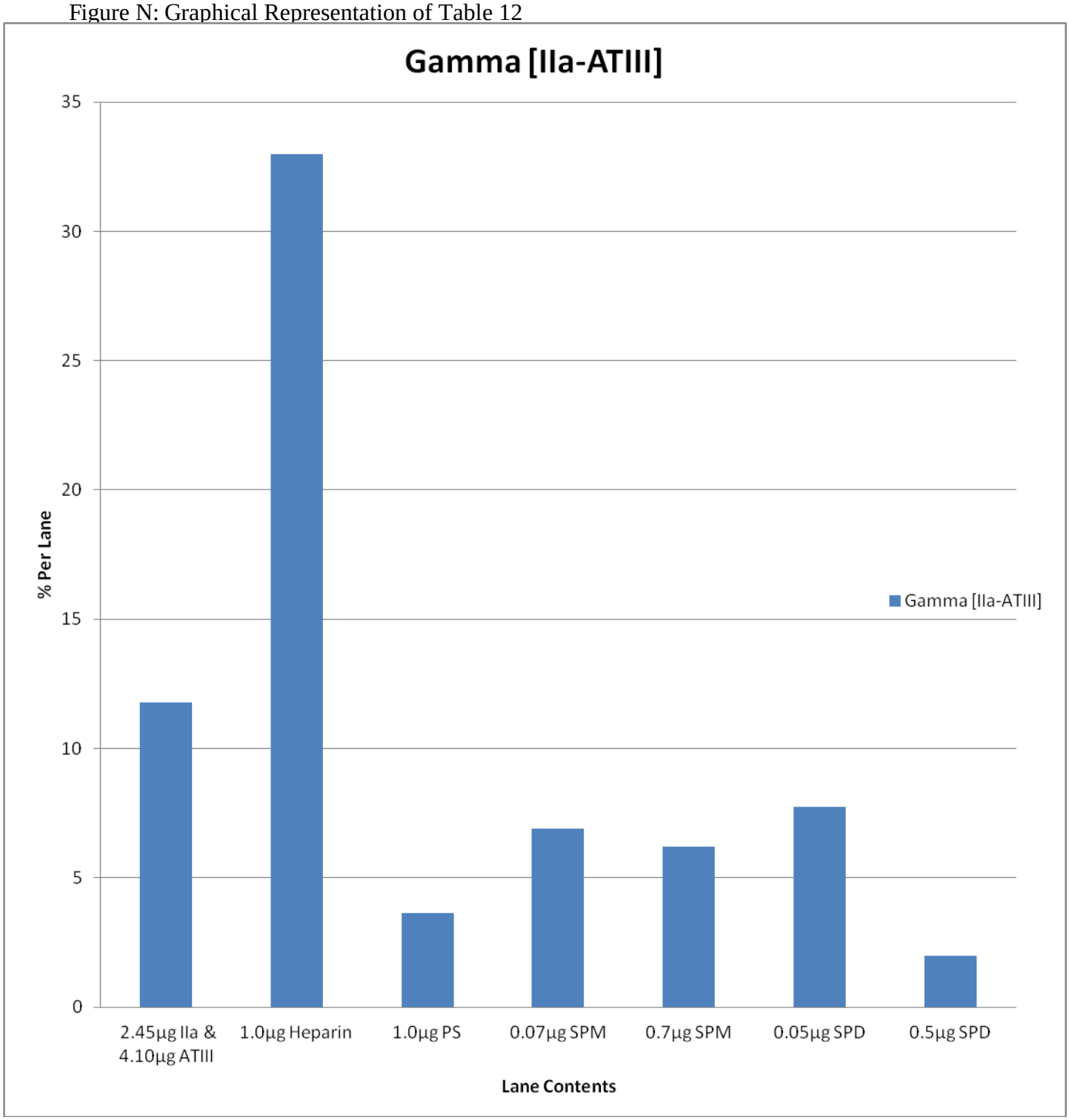


Table 13: ATIII-M Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIIIM	2.82	4.47	0.8	0.54	2.04	0.44	0.49

Figure O: Graphical Representation of Table 13

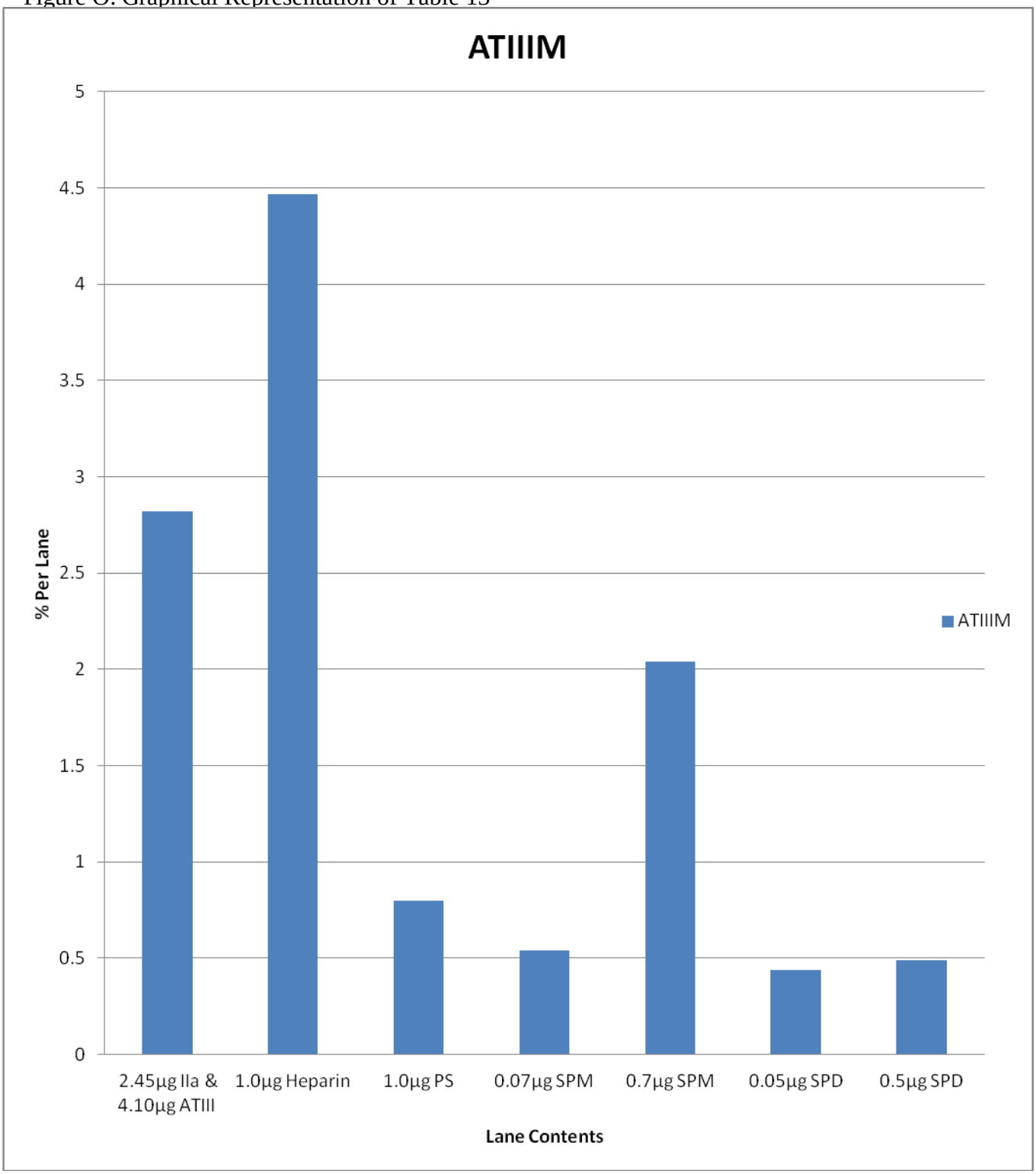


Table 14: ATIII Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIII	3.73	5.16	2.8	4.12	5.08	3.75	5.93

Figure P: Graphical Representation of Table 14

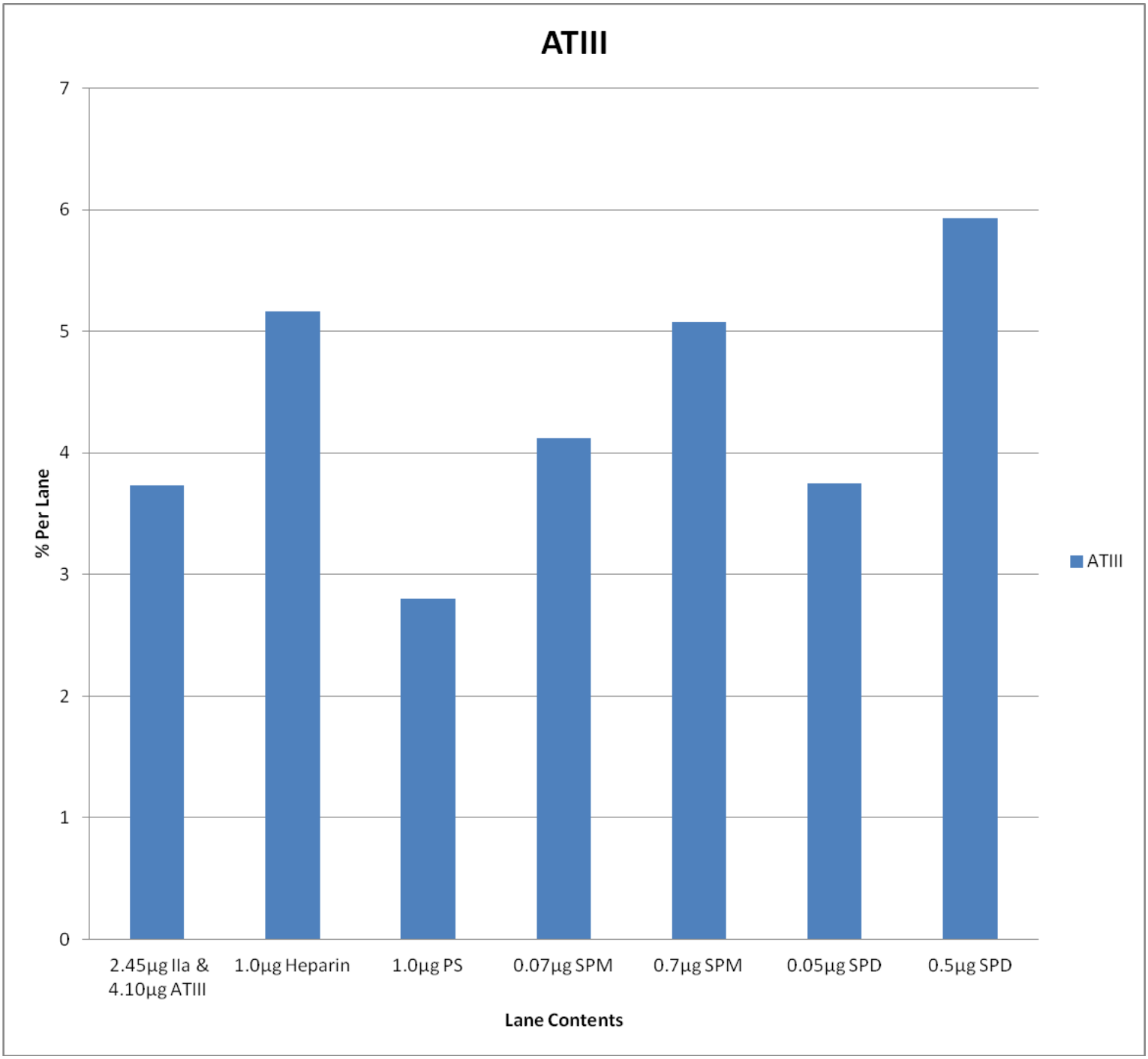


Table 15 IIa Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
IIa	25.9	30.5	20.5	15.7	23.7	23.4	6.52

Figure Q: Graphical Representation of Table 15

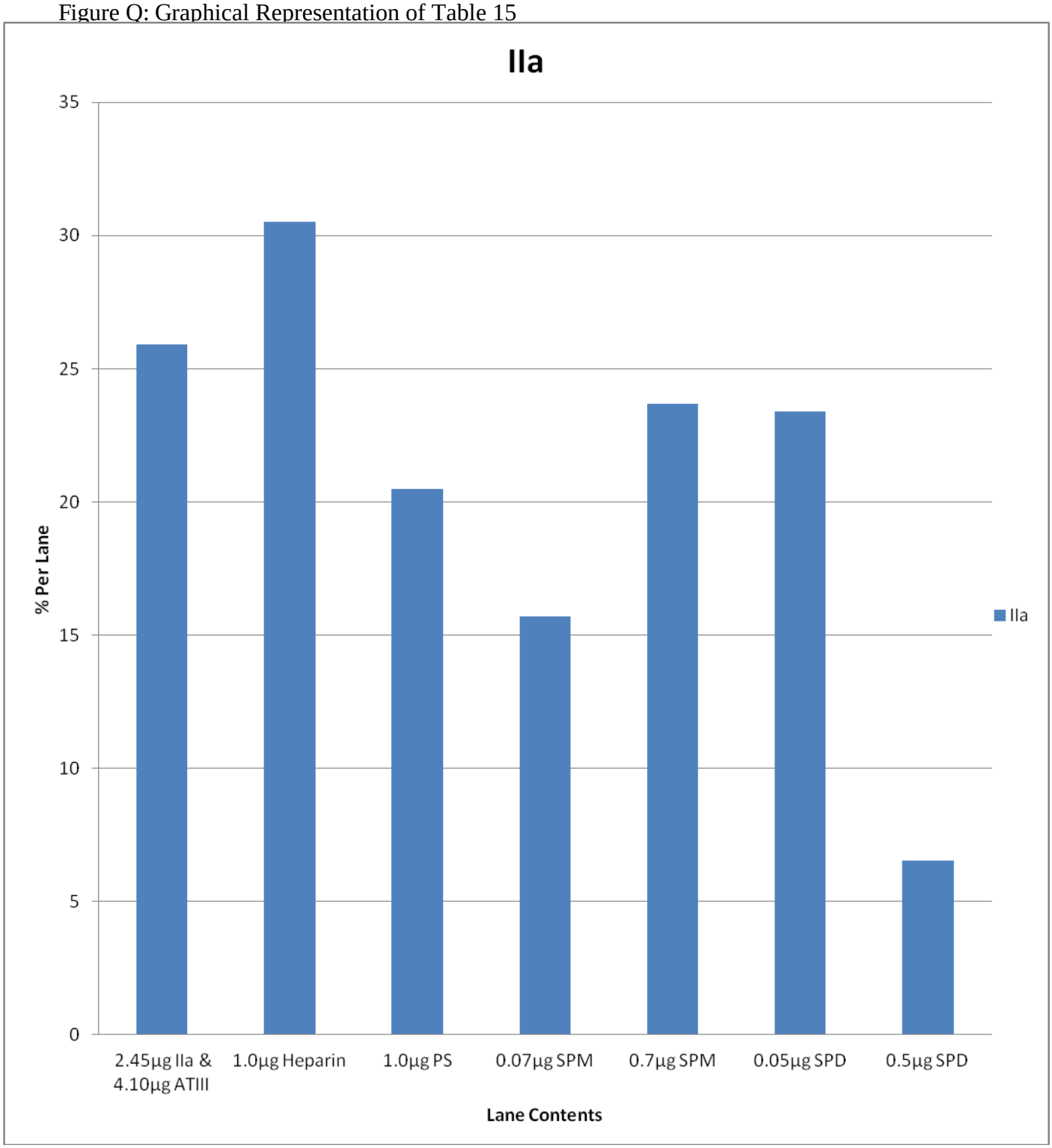
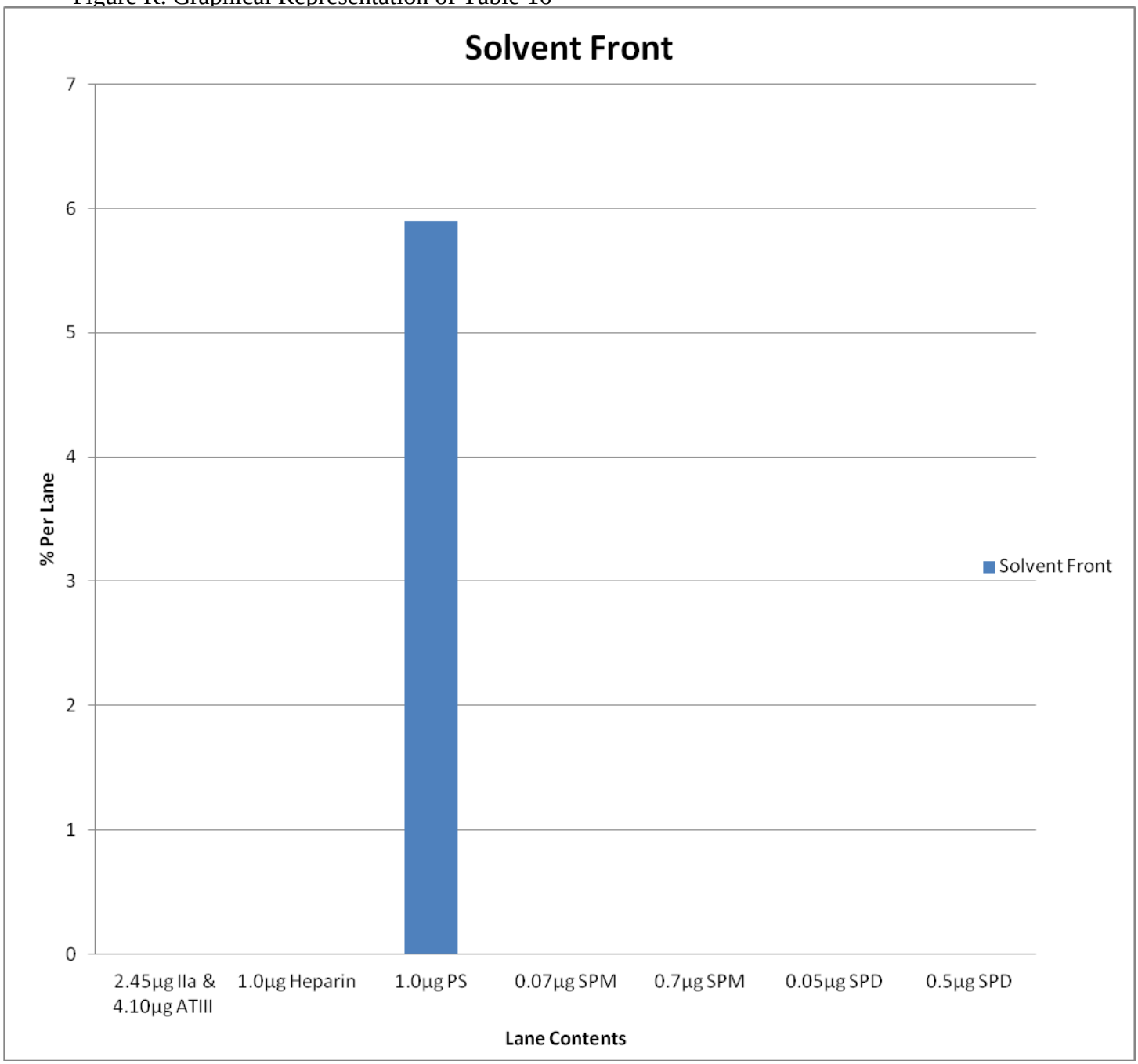


Table 16: Solvent Front Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Solvent Front	*ND	*ND	5.9	*ND	*ND	*ND	*ND

*ND=Not Detected

Figure R: Graphical Representation of Table 16



Gel 5 & Gel 6

Figure S: Gel 5

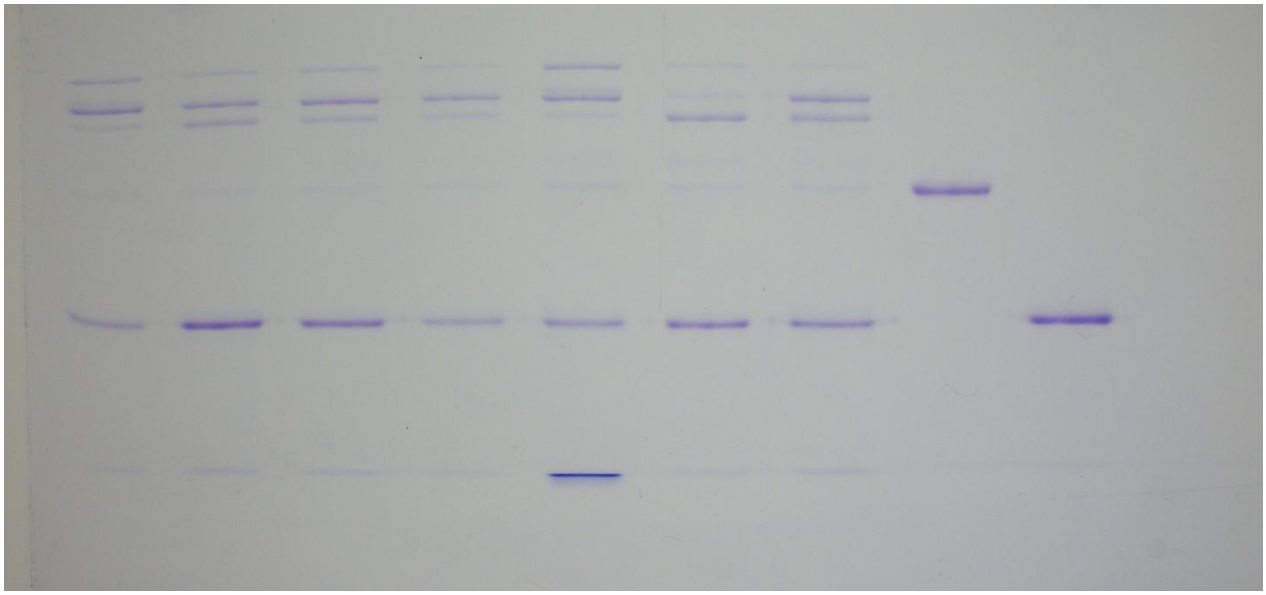
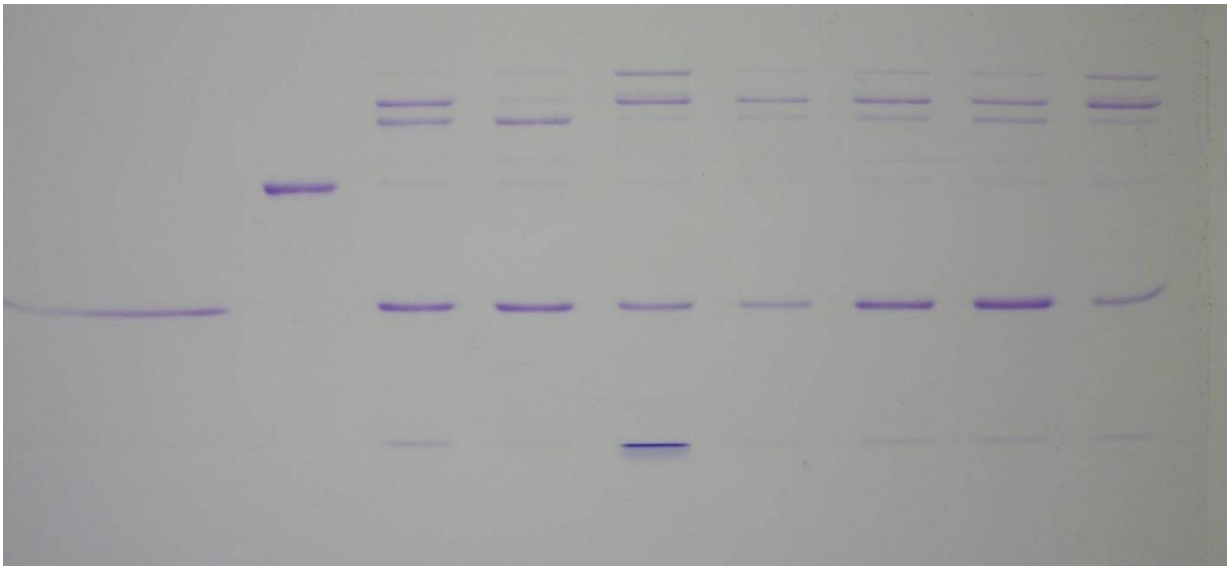


Figure T: Gel 6



Gel 5

Table 17: Overall Protein Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Alpha [IIa-ATIII]	1.58	3.54	12.9	8.19	6.17	4.25	15.9
Beta [IIa-ATIIII]	27.2	2.65	20.4	32.4	26.8	20.2	40.4
Gamma [IIa-ATIII]	19.5	27	2.31	9.01	9.74	12.1	5.7
ATIIIM	1.12	1.23	0.31	2.36	0.81	0.4	2.41
ATIII	1.3	3.28	1.5	2.9	2.27	2.51	4.06
IIa	44.9	57.4	23.9	42.2	49.9	57.1	27.9
Solvent Front	4.42	4.85	38.6	2.93	4.35	3.4	3.63

Standard Eviation	16.35839201	Mean	14.2832653	Standard Error	2.336913

Figure U: Graphical Representation of Table 17

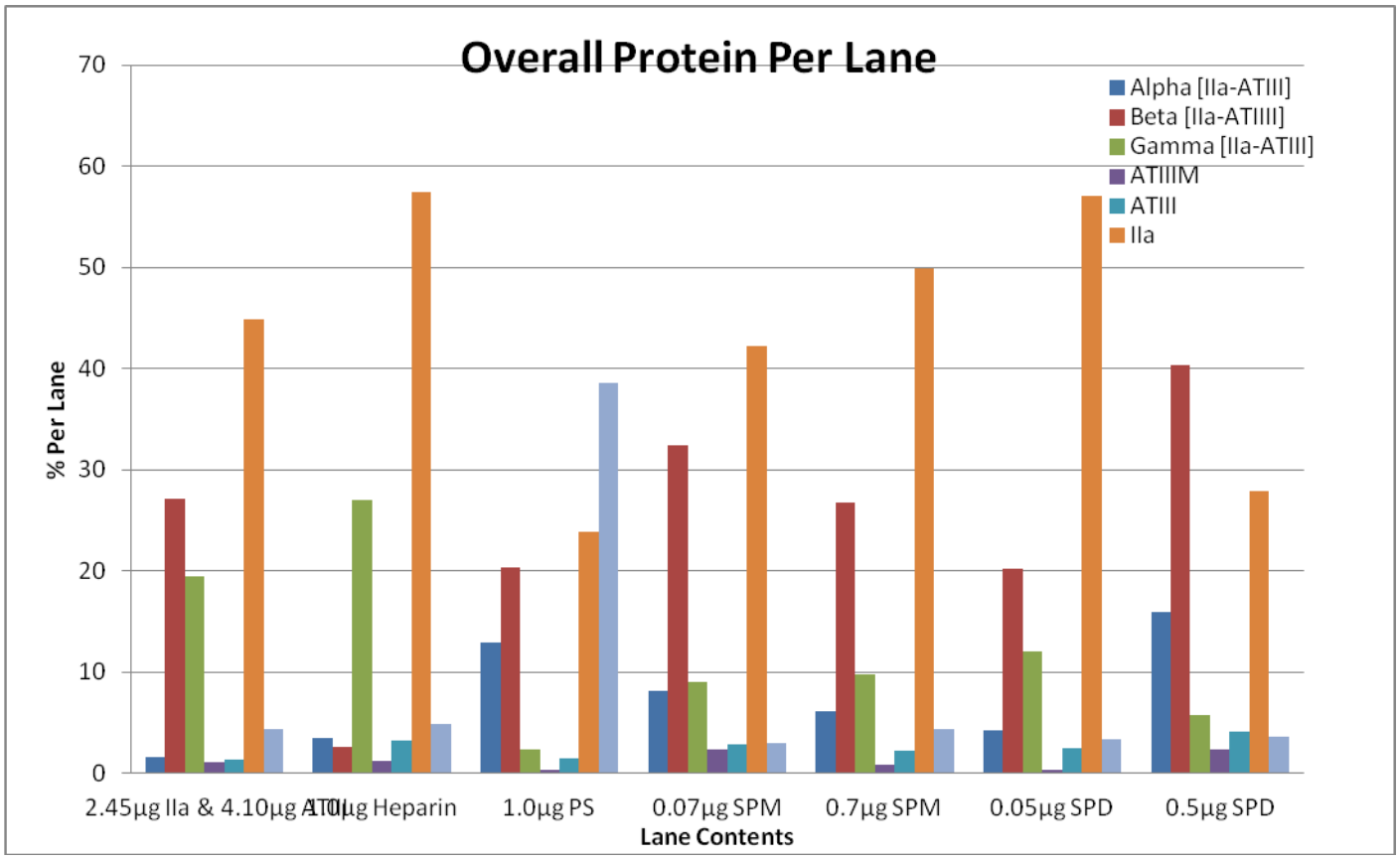


Table 18: Alpha [IIa-ATIII] Complex Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Alpha [IIa-ATIII]	1.58	3.54	12.9	8.19	6.17	4.25	15.9

Figure V: Graphical Representation of Table 18

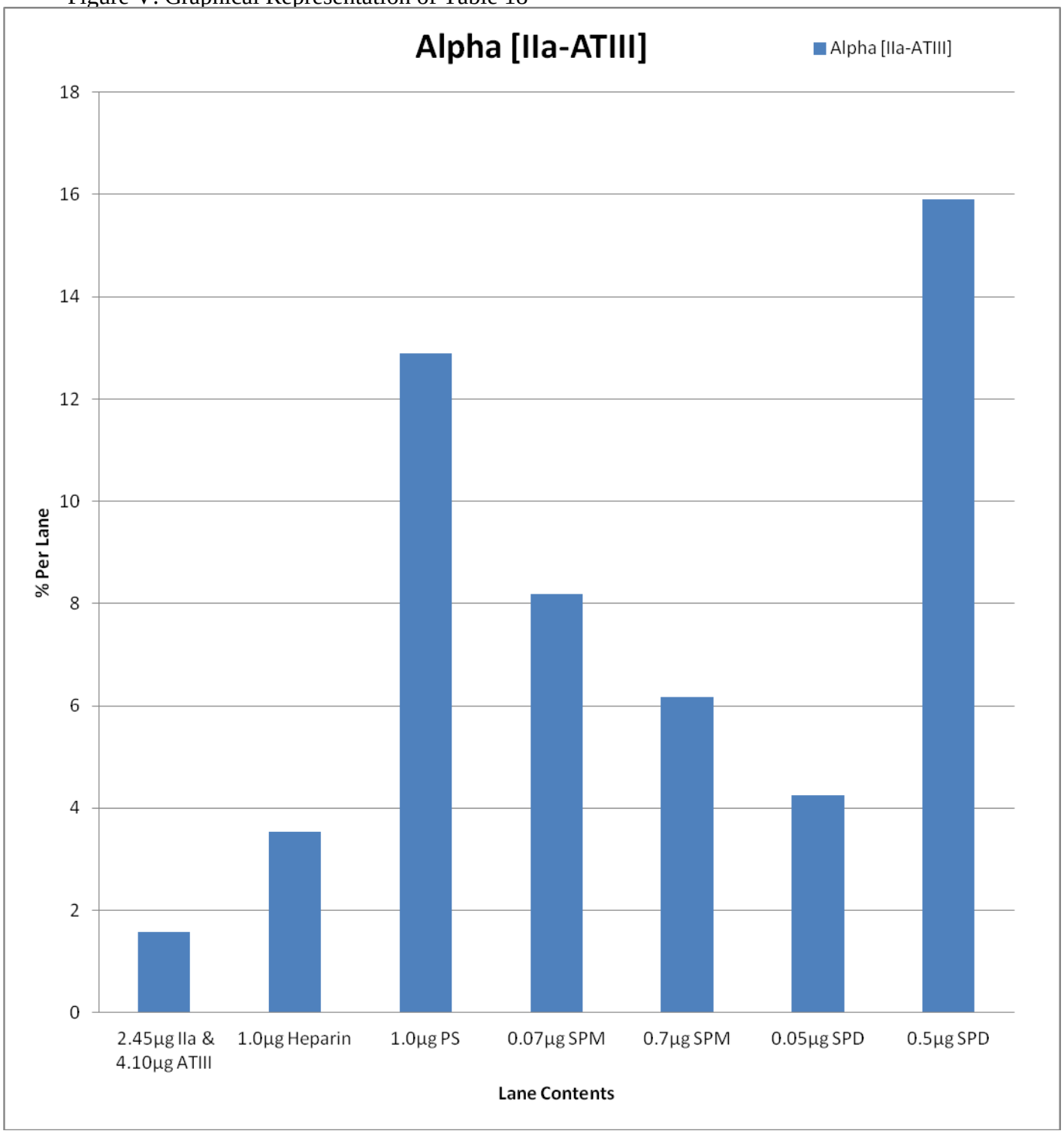


Table 19: Beta [IIa-ATIII] Complex Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Beta [IIa-ATIIII]	27.2	2.65	20.4	32.4	26.8	20.2	40.4

Figure W: Graphical Representation of Table 19

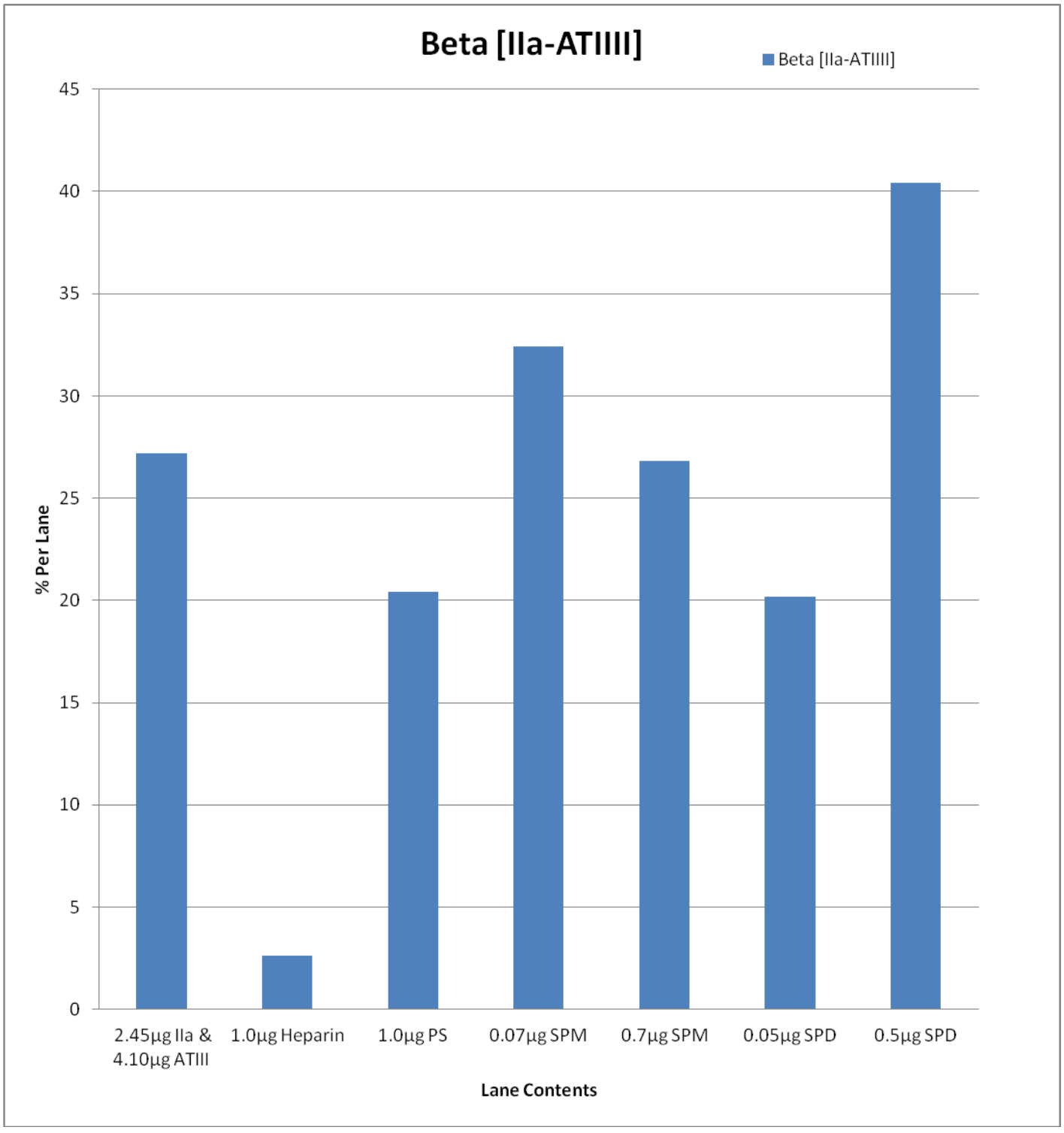


Table 20: Gamma [IIa-ATIII] Complex Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Gamma [IIa-ATIII]	19.5	27	2.31	9.01	9.74	12.1	5.7

Figure X: Graphical Representation of Table 20

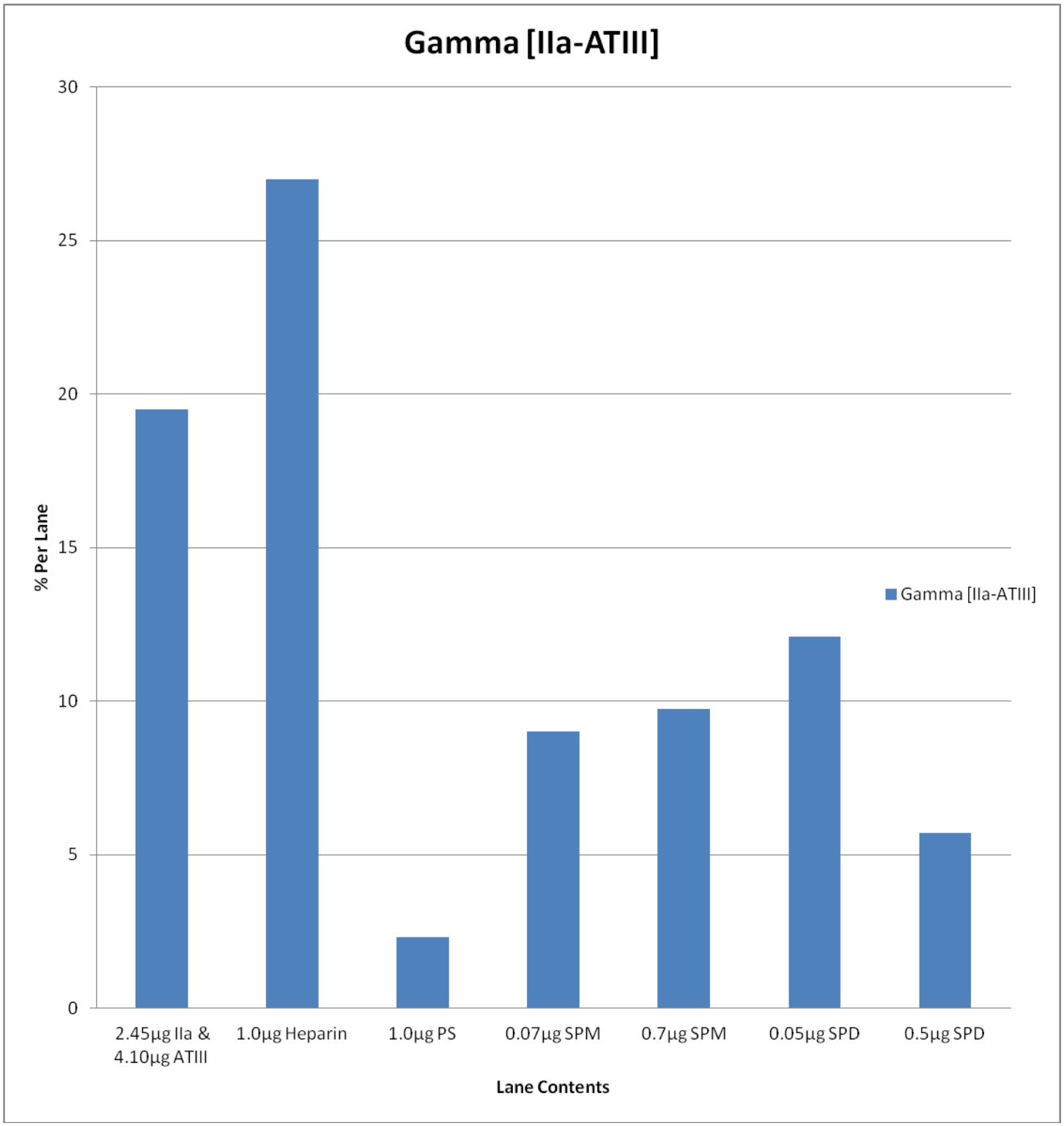


Table 21: ATIII-M Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIIIM	1.12	1.23	0.31	2.36	0.81	0.4	2.41

Figure Y: Graphical Representation of Table 21

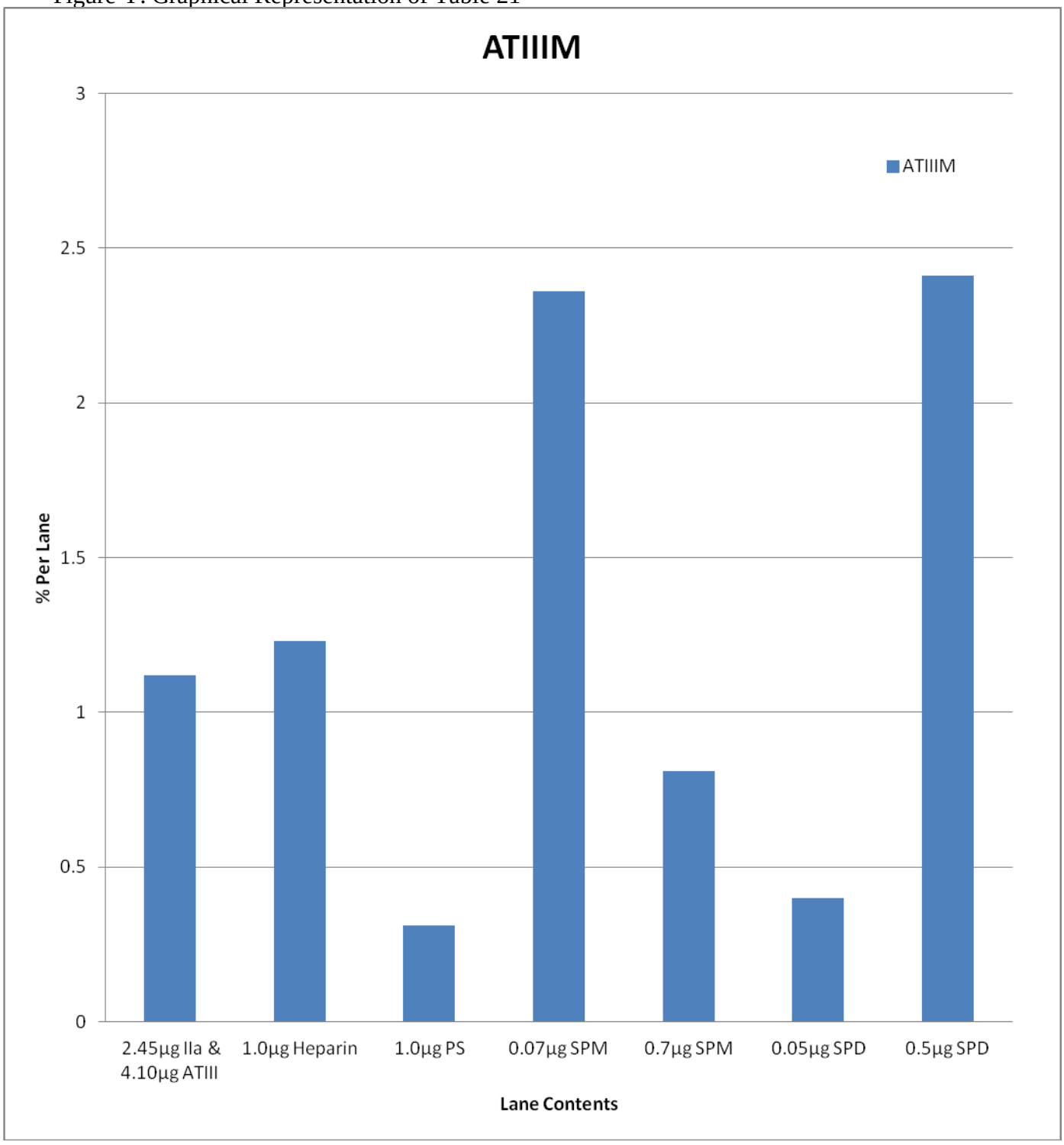


Table 22: ATIII Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIII	1.3	3.28	1.5	2.9	2.27	2.51	4.06

Figure Z: Graphical Representation of Table 22

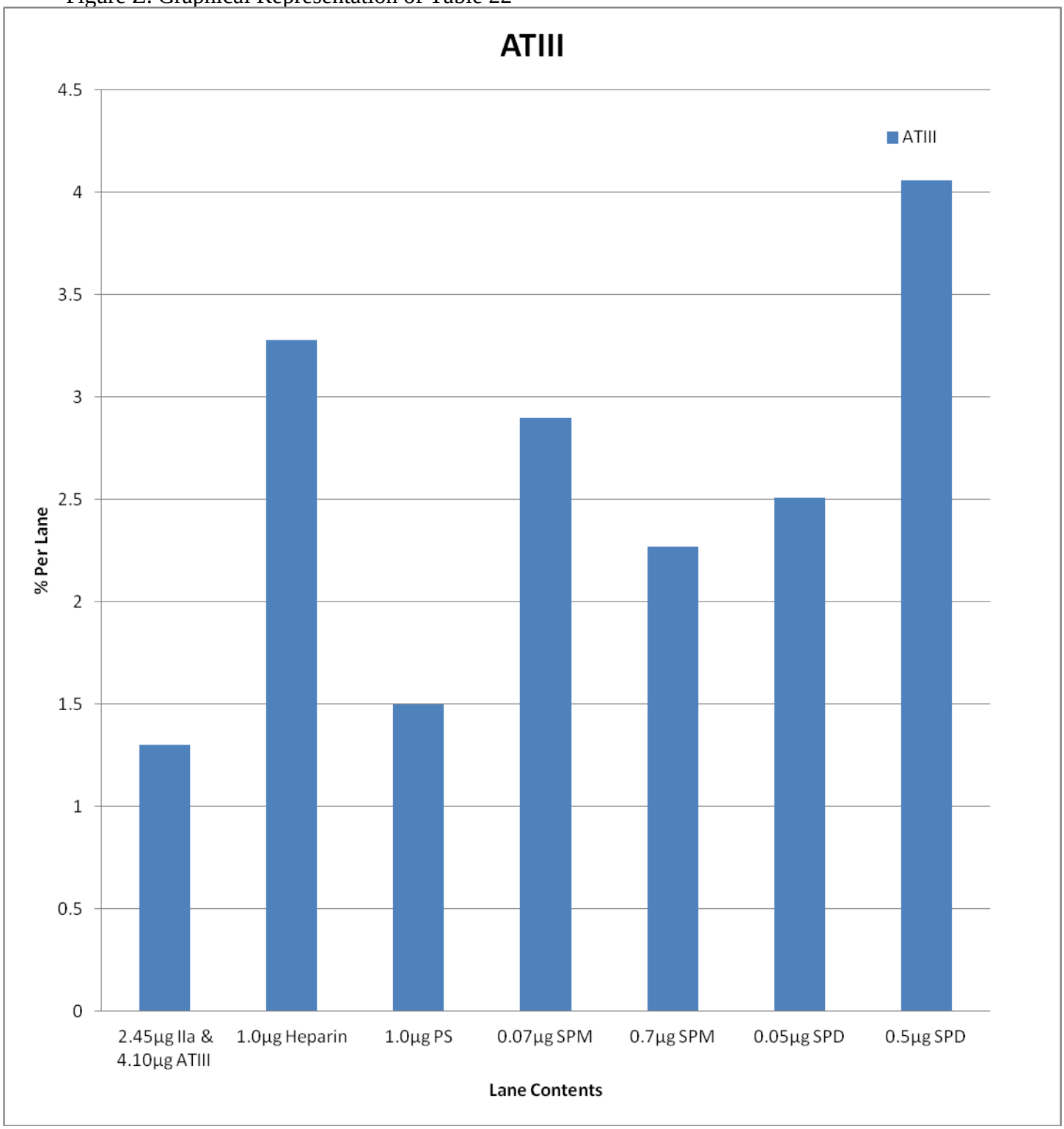


Table 23: IIa Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
IIa	44.9	57.4	23.9	42.2	49.9	57.1	27.9

Figure AA: Graphical Representation of Table 23

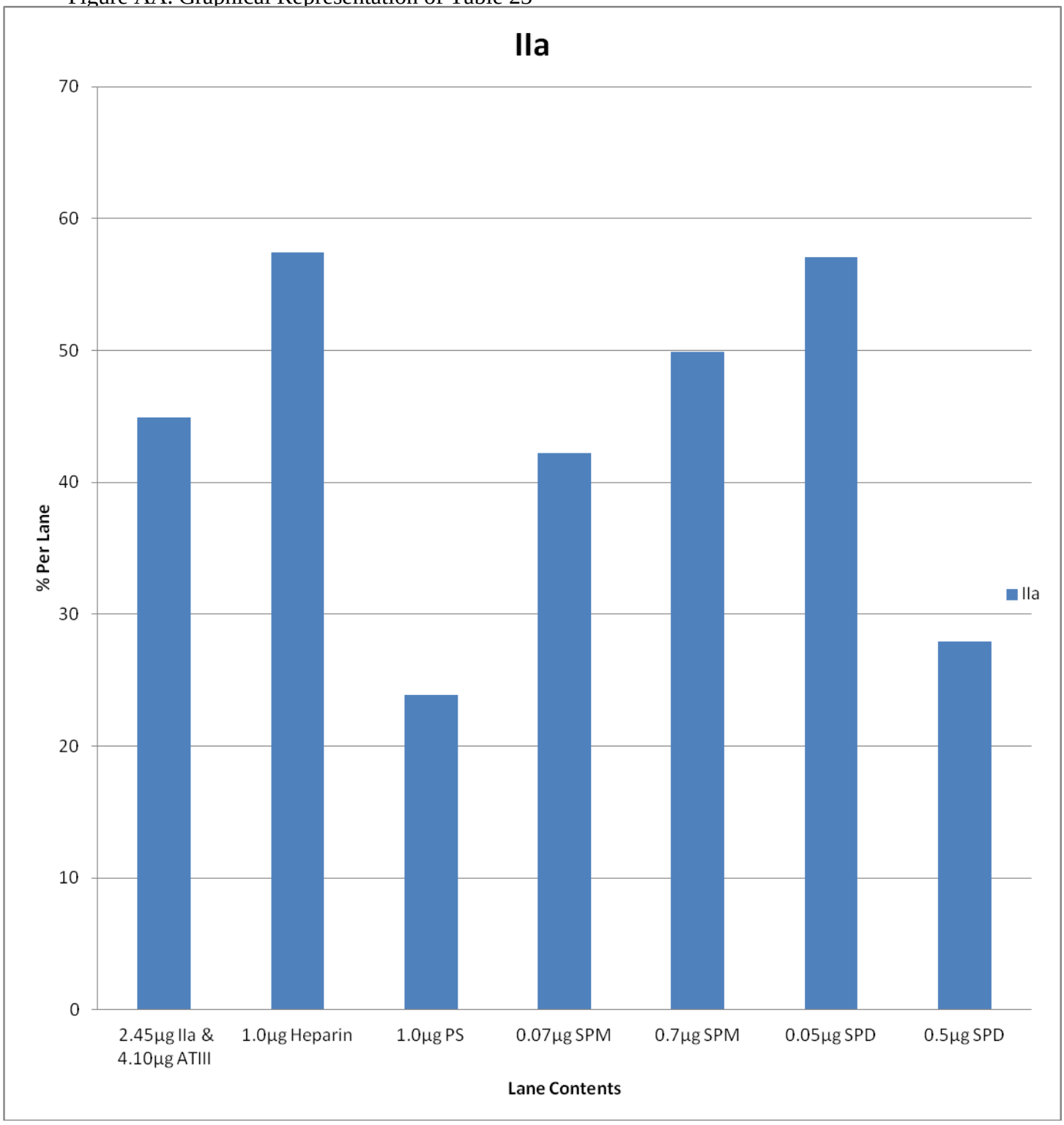
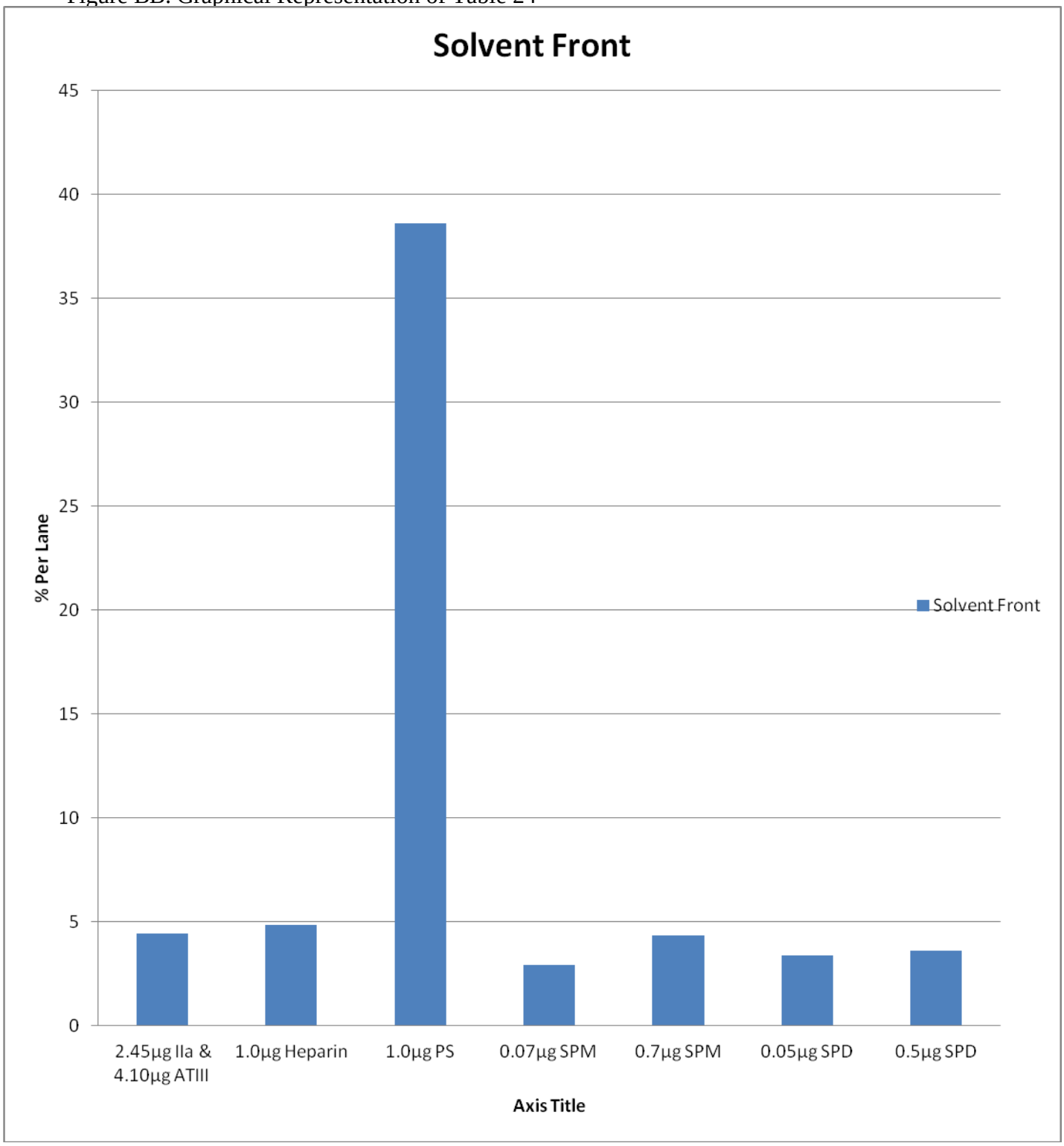


Table 24: Solvent Front Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Solvent Front	4.42	4.85	38.6	2.93	4.35	3.4	3.63

Figure BB: Graphical Representation of Table 24



Gel 6

Table 25: Overall Protein Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Alpha [IIa-ATIII]	1.48	2.2	12.1	3.44	4.51	3.62	16
Beta [IIa-ATIIII]	25.1	2.32	18.9	34.7	23	16.4	39.9
Gamma [IIa-ATIII]	18.7	28.7	1.94	9.4	8.65	9.6	8.09
ATIIIM	0.46	3.03	0.28	2.48	0.53	0.72	1.13
ATIII	1.63	2.86	1.21	3.29	1.67	1.31	3.58
IIa	46.9	58.5	22.7	45.4	56.4	64.1	24.7
Solvent Front	5.8	2.38	42.8	1.26	5.32	4.33	6.61

Standard Deviation	18.43506082	Mean	14.2883673	Standard Error	2.63358

Figure CC: Graphical Representation of Table 25

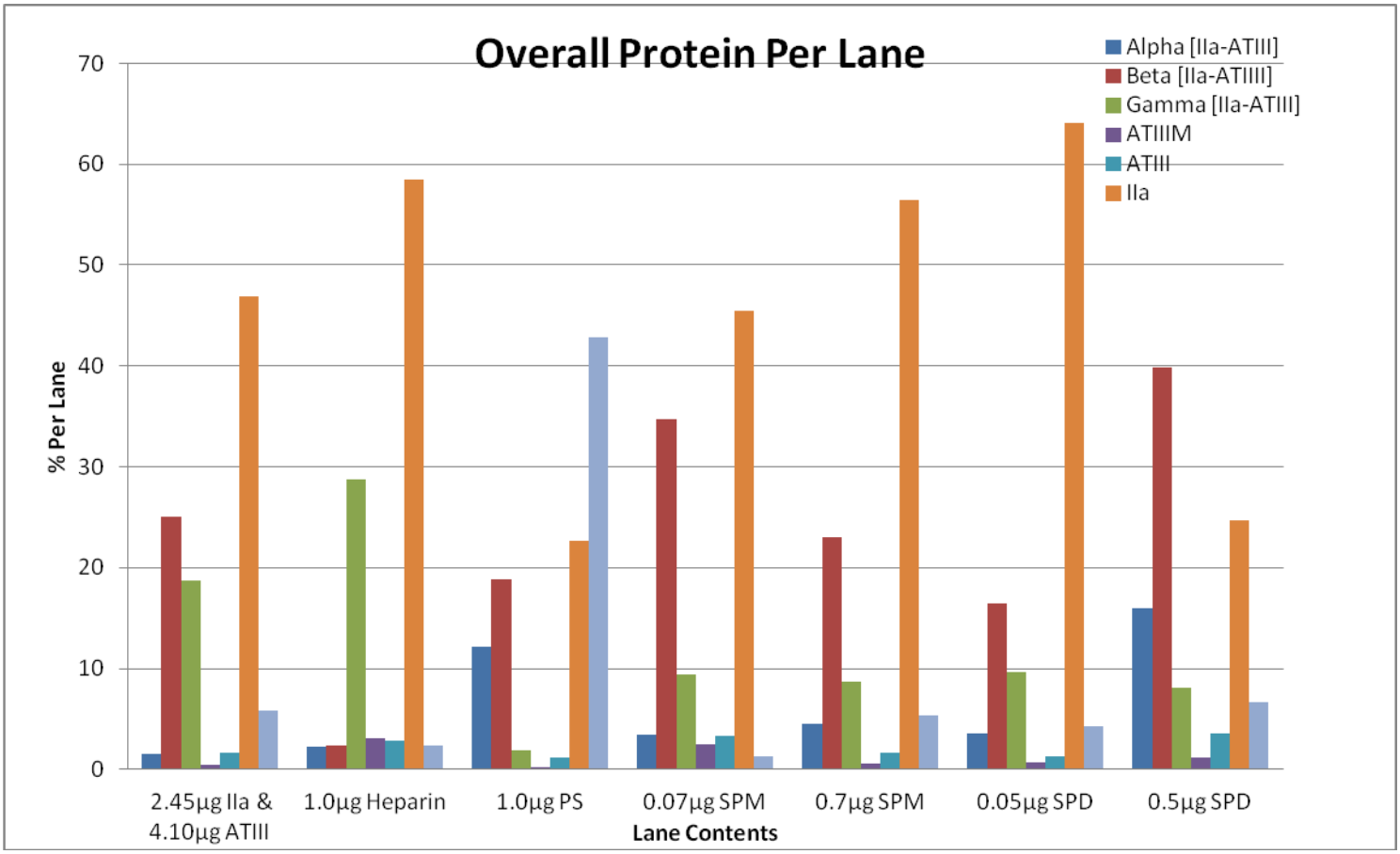


Table 26: -Alpha [IIa-ATIII] Complex Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Alpha [IIa-ATIII]	1.48	2.2	12.1	3.44	4.51	3.62	16

Figure DD: Graphical Representation of Table 26

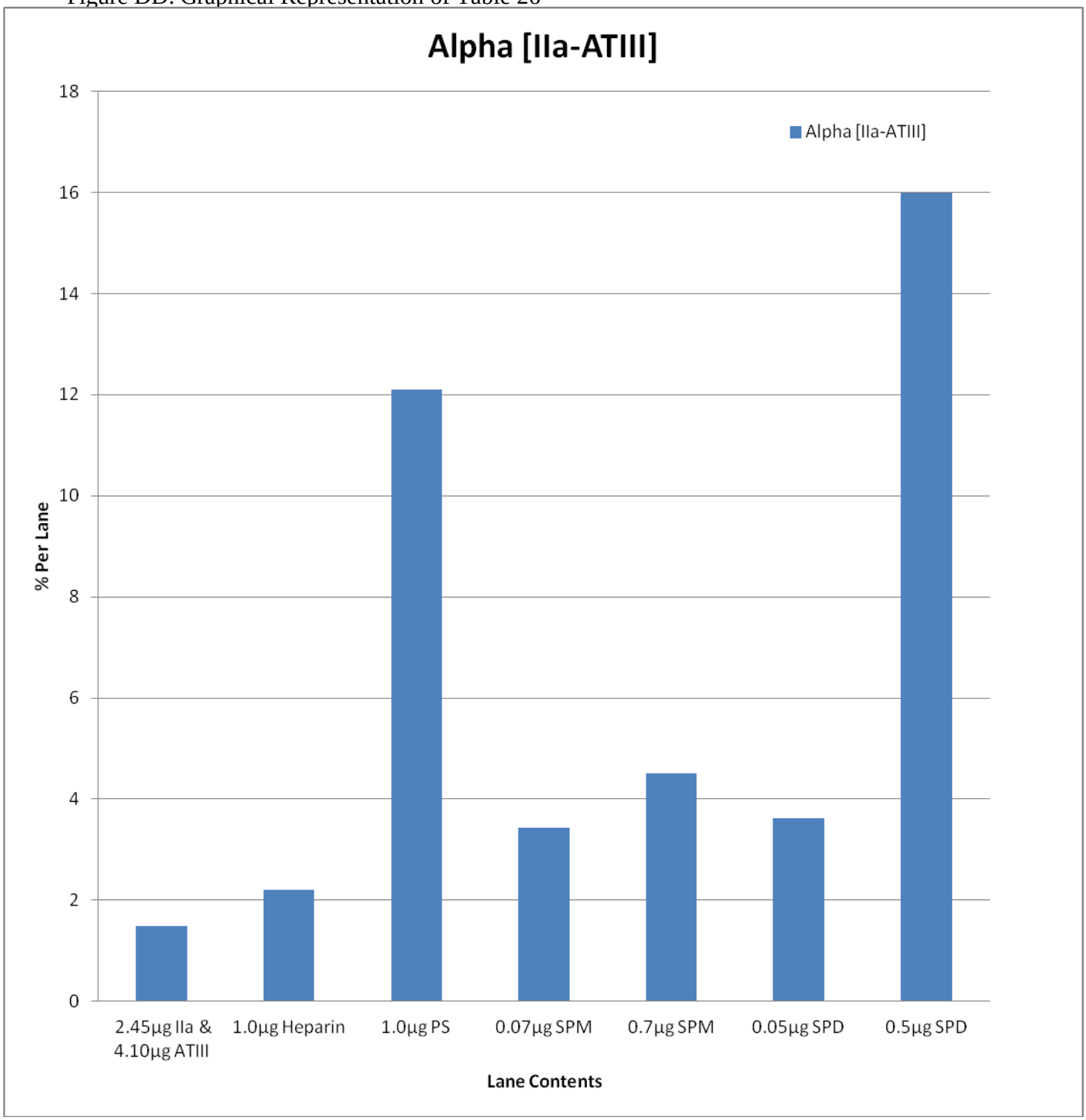


Table 27: Beta [IIa-ATIII] Complex Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Beta [IIa-ATIIII]	25.1	2.32	18.9	34.7	23	16.4	39.9

Figure EE: Graphical Representation of Table 27

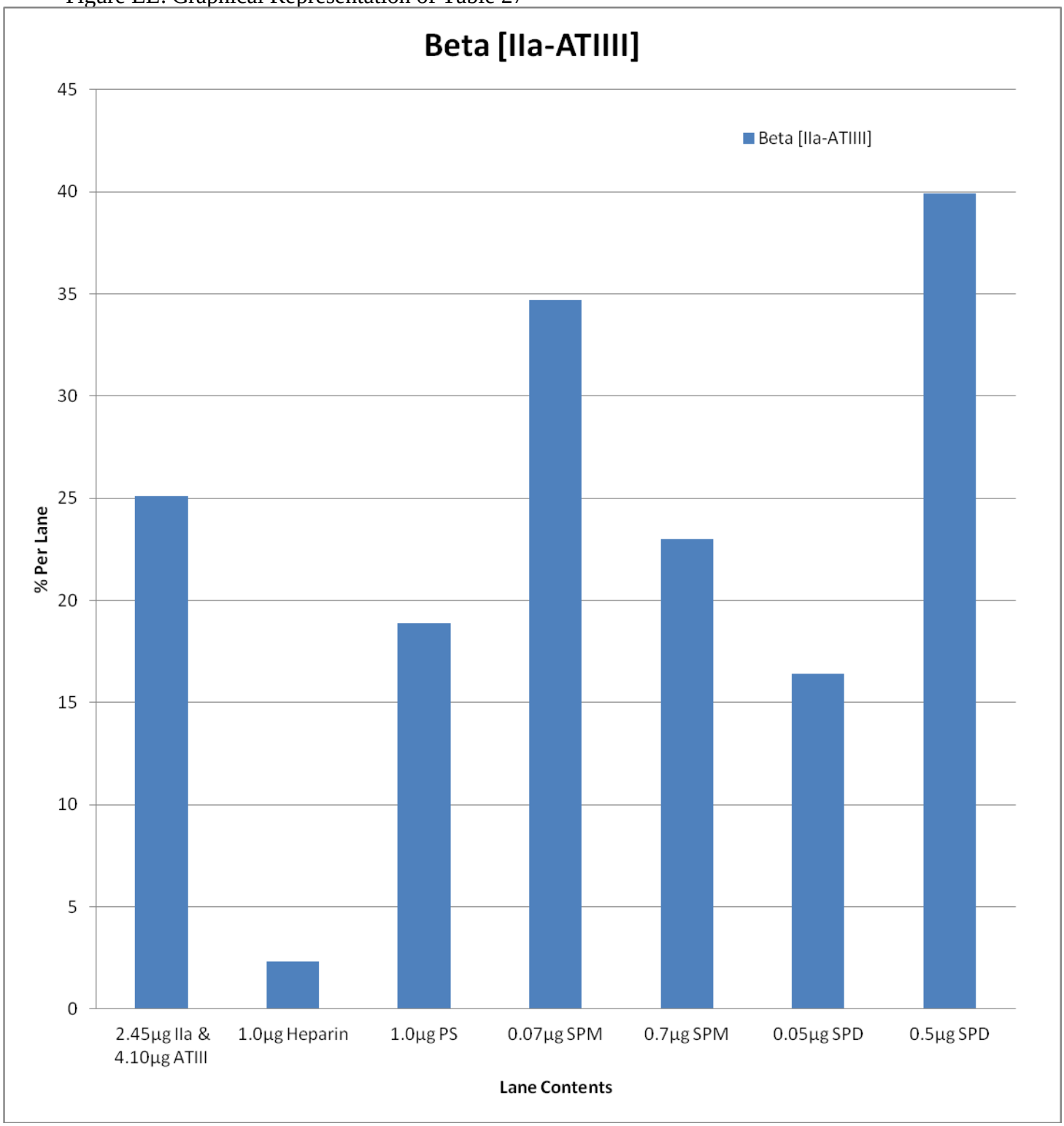


Table 28: Gamma [IIa-ATIII] Complex Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Gamma [IIa-ATIII]	18.7	28.7	1.94	9.4	8.65	9.6	8.09

Figure FF: Graphical Representation of Table 28

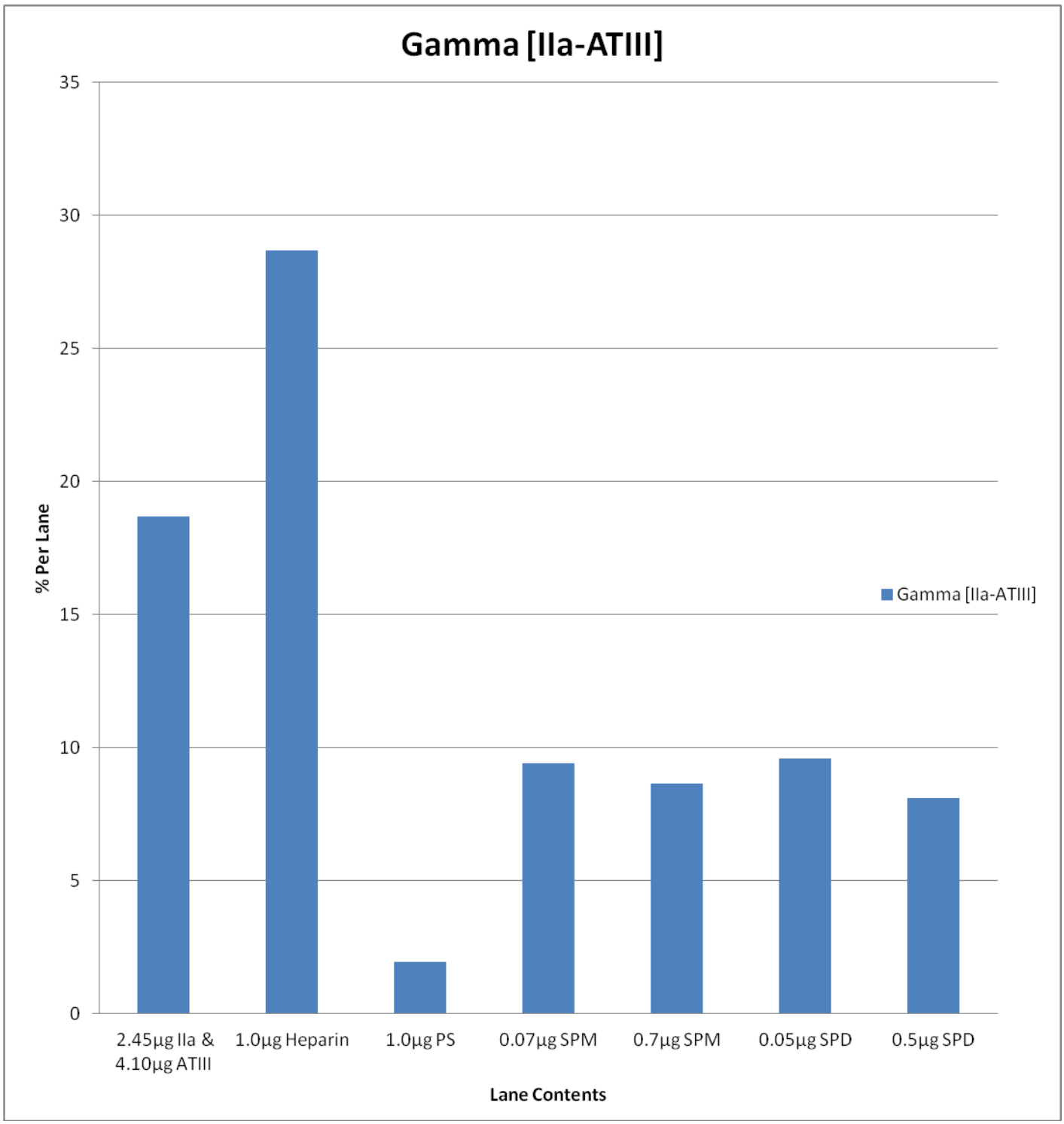


Table 29: ATIII-M Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIIIM	0.46	3.03	0.28	2.48	0.53	0.72	1.13

Figure GG: Graphical Representation of Table 29

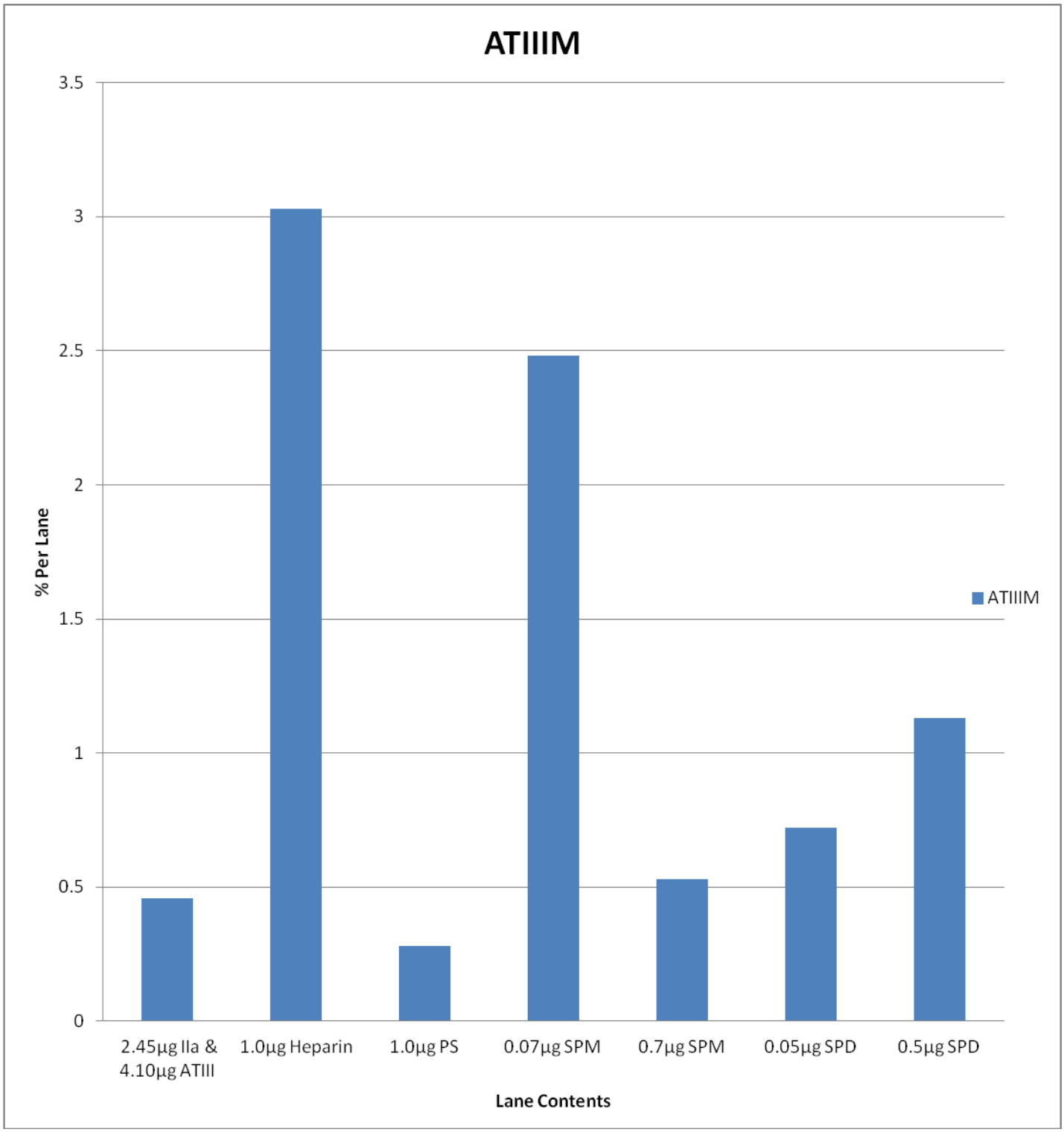


Table 30: ATIII Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
ATIII	1.63	2.86	1.21	3.29	1.67	1.31	3.58

Figure HH: Graphical Representation of Table 30

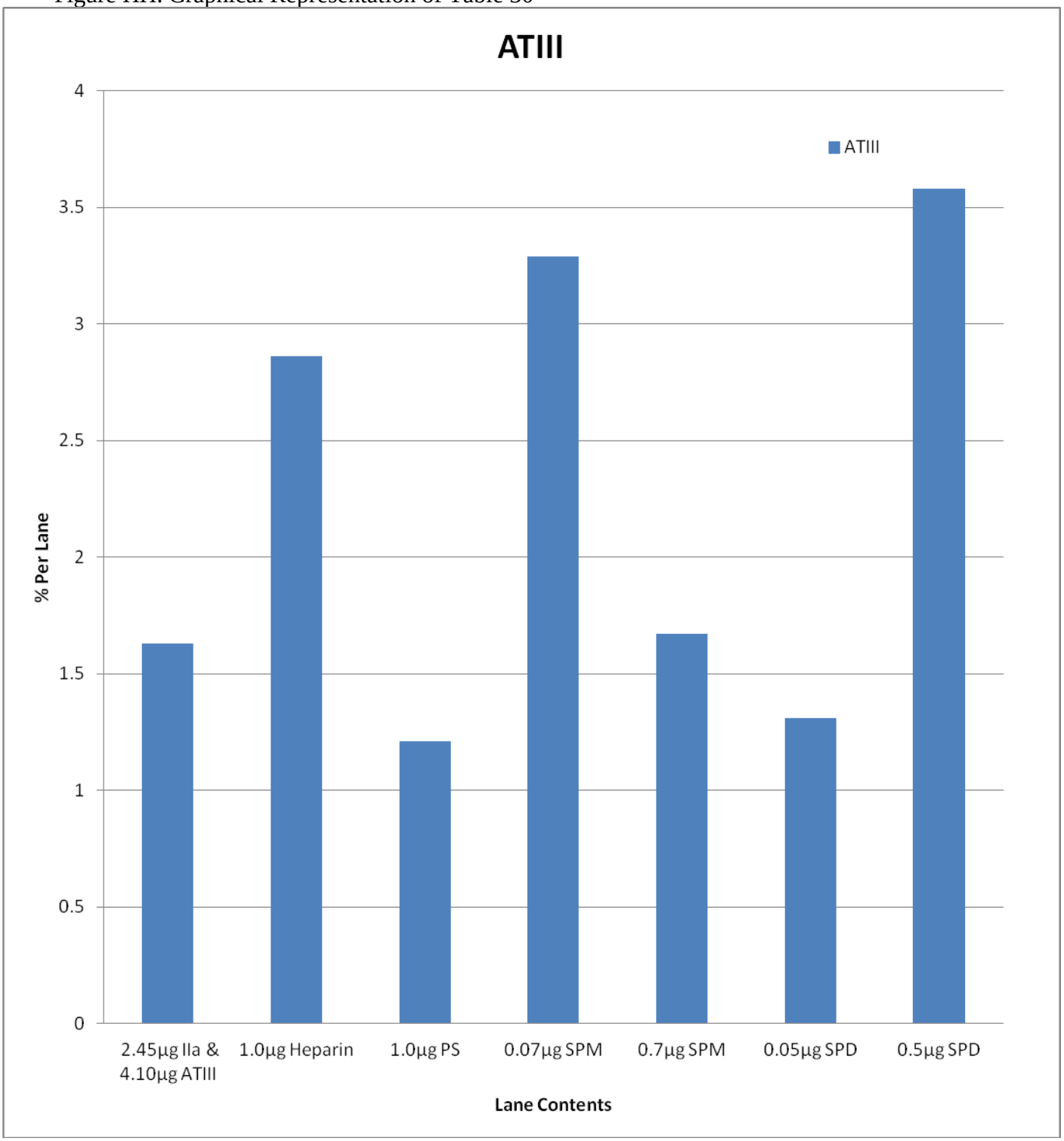


Table 31: IIa Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
IIa	46.9	58.5	22.7	45.4	56.4	64.1	24.7

Figure II: Graphical Representation of Table 31

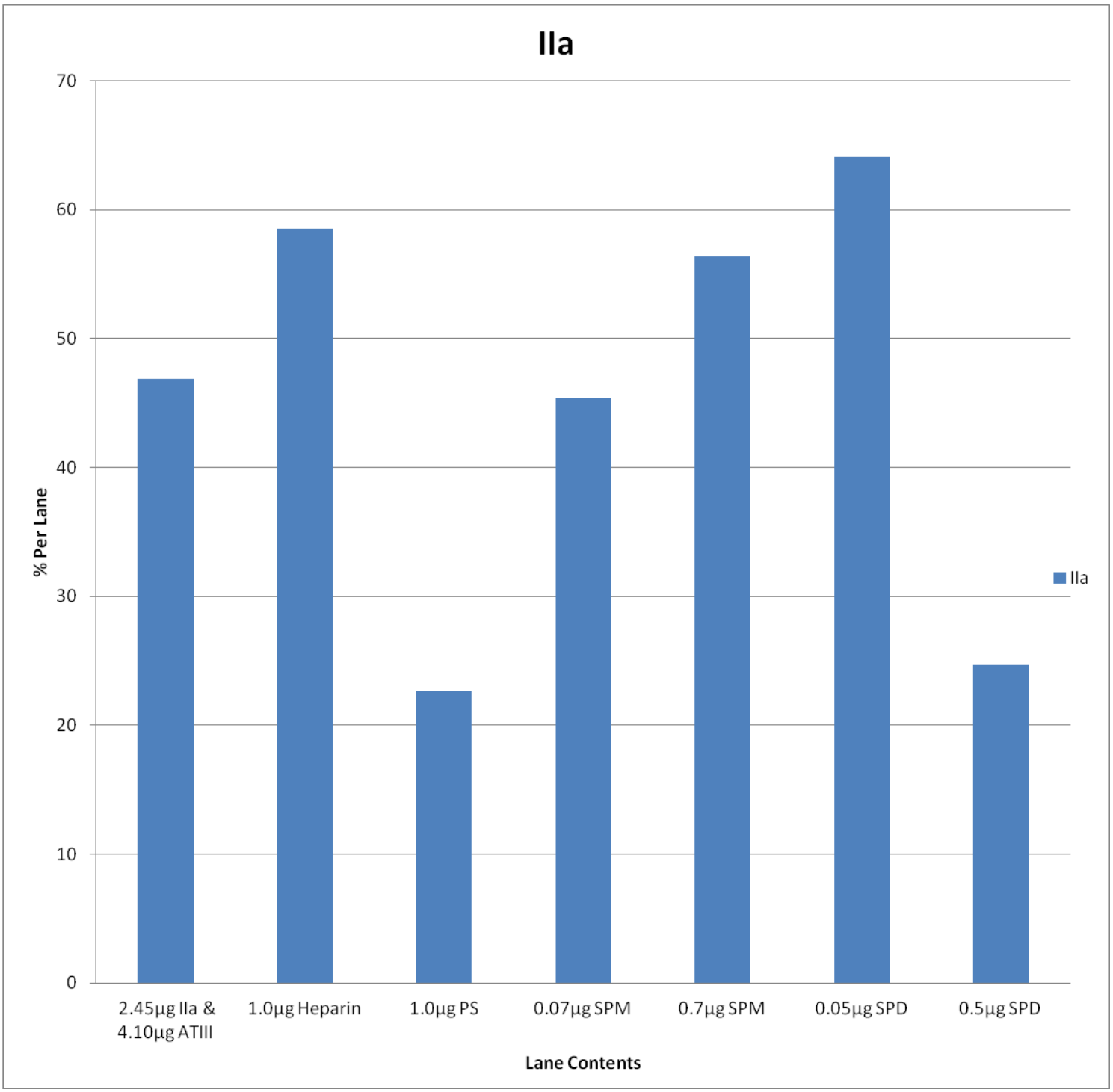
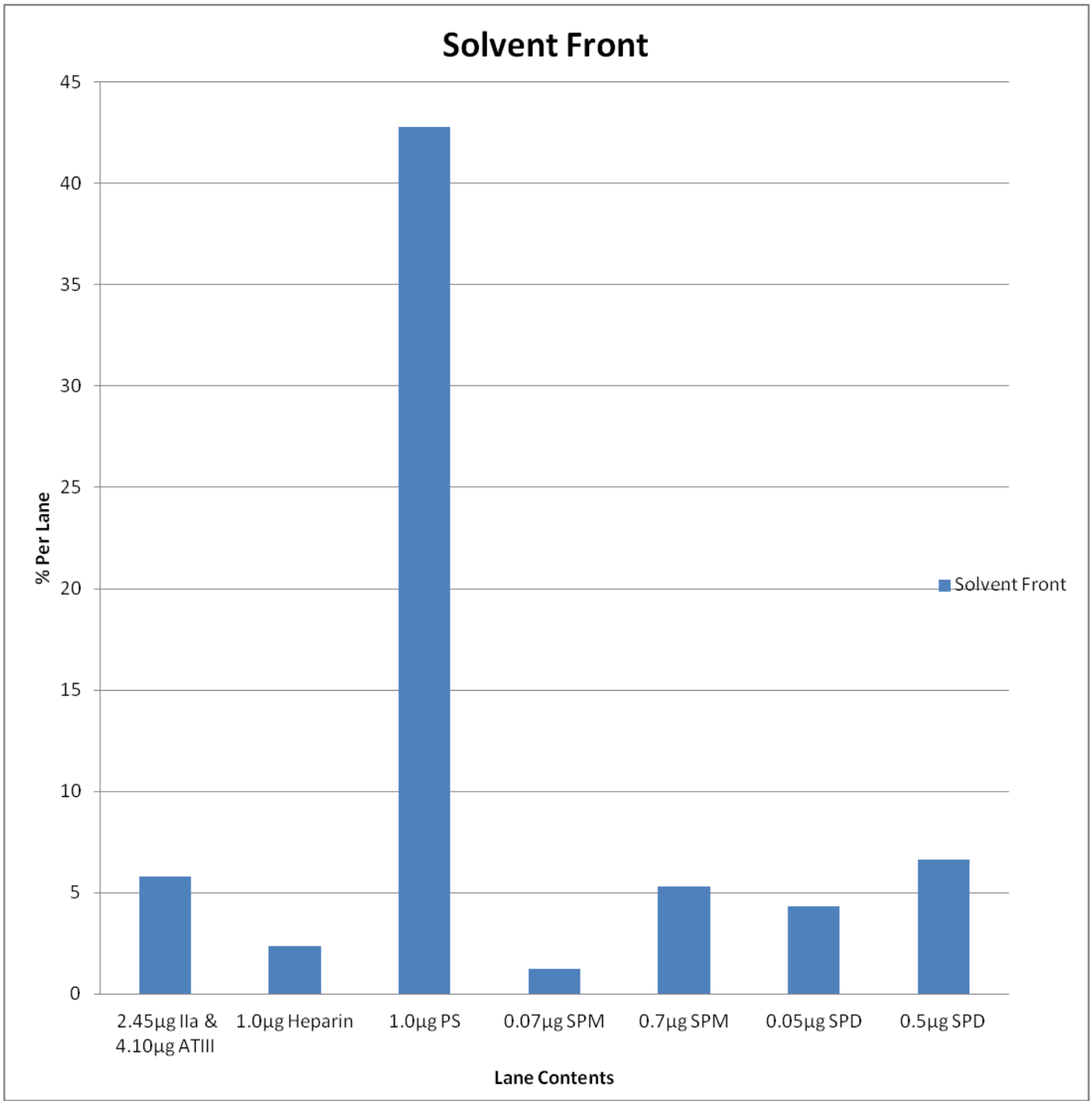


Table 32: Solvent Front Analysis Per Lane

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Solvent Front	5.8	2.38	42.8	1.26	5.32	4.33	6.61

Figure JJ: Graphical Representation of Table 32



Tables used for P Value Analysis

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Alpha [IIa-ATIII]	13.8	6.4	37.7	34.2	24.7	24.8	51.9
Alpha [IIa-ATIII]	13.7	5.88	35.7	32.9	24	23.2	54.1
Alpha [IIa-ATIII]	1.58	3.54	12.9	8.19	6.17	4.25	15.9
Alpha [IIa-ATIII]	1.48	2.2	12.1	3.44	4.51	3.62	16

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Beta [IIa-ATIIII]	36.7	21.7	30.3	39.1	36.4	42.1	32.1
Beta [IIa-ATIIII]	42.1	21	30.6	39.8	39	41.5	30.9
Beta [IIa-ATIIII]	27.2	2.65	20.4	32.4	26.8	20.2	40.4
Beta [IIa-ATIIII]	25.1	2.32	18.9	34.7	23	16.4	39.9

	2.45µg IIa & 4.10µg ATIII	1.0µg Heparin	1.0µg PS	0.07µg SPM	0.7µg SPM	0.05µg SPD	0.5µg SPD
Gamma [IIa-ATIII]	12.3	34.2	4.24	6.9	9.36	8.27	2.59
Gamma [IIa-ATIII]	11.8	33	3.65	6.92	6.21	7.75	2
Gamma [IIa-ATIII]	19.5	27	2.31	9.01	9.74	12.1	5.7
Gamma [IIa-ATIII]	18.7	28.7	1.94	9.4	8.65	9.6	8.09

Alpha Complex P Values

Alpha [IIa-ATIII]

	2.45µg IIa & 4.10µg ATIII	*1.0µg Heparin*
Mean	7.64	4.505
Variance	49.77946667	3.908633333
t Stat	1.206963841	
P(T<=t) one-tail	0.156966904	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.313933809	
t Critical two-tail	3.182446305	

	2.45µg IIa & 4.10µg ATIII	*1.0µg PS*
Mean	7.64	24.6
Variance	49.77946667	195.9866667
t Stat	-4.869450252	
P(T<=t) one-tail	0.008273745	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.016547489	
t Critical two-tail	3.182446305	

	2.45µg IIa & 4.10µg ATIII	*0.07µg SPM*
Mean	7.64	19.6825
Variance	49.77946667	260.4521583
t Stat	-2.626604748	
P(T<=t) one-tail	0.039276975	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.07855395	
t Critical two-tail	3.182446305	

	2.45µg IIa & 4.10µg ATIII	*0.7µg SPM*
Mean	7.64	14.845
Variance	49.77946667	121.0009667
t Stat	-3.621376583	
P(T<=t) one-tail	0.018106151	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.036212302	
t Critical two-tail	3.182446305	

	2.45µg IIa & 4.10µg ATIII	*0.05µg SPD*
Mean	7.64	13.9675

Variance	49.77946667	134.694225
t Stat	-2.765732641	
P(T<=t) one-tail	0.034908445	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.069816889	
t Critical two-tail	3.182446305	

	2.45µg IIa & 4.10µg ATIII	*0.5µg SPD*
Mean	7.64	34.475
Variance	49.77946667	458.3758333
t Stat	-3.735759315	
P(T<=t) one-tail	0.016720981	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.033441963	
t Critical two-tail	3.182446305	

Beta Complex P Values

Beta [IIa-ATIII]

	2.45µg IIa & 4.10µg ATIII	*1.0µg Heparin*
Mean	32.775	11.9175
Variance	64.11583333	118.729225
t Stat	10.0486789	
P(T<=t) one-tail	0.001049163	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.002098327	
t Critical two-tail	3.182446305	

	2.45µg IIa & 4.10µg ATIII	*1.0µg PS*
Mean	32.775	25.05
Variance	64.11583333	39.27
t Stat	6.109137707	
P(T<=t) one-tail	0.004406754	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.008813508	
t Critical two-tail	3.182446305	

	2.45µg IIa & 4.10µg ATIII	*0.07µg SPM*
Mean	32.775	36.5
Variance	64.11583333	12.56666667
t Stat	-1.492514518	
P(T<=t) one-tail	0.116194171	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.232388342	
t Critical two-tail	3.182446305	

	2.45µg IIa & 4.10µg ATIII	*0.7µg SPM*
Mean	32.775	31.3
Variance	64.11583333	58.14666667
t Stat	2.16547179	
P(T<=t) one-tail	0.059480749	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.118961498	
t Critical two-tail	3.182446305	

	2.45µg IIa & 4.10µg ATIII	*0.05µg SPD*
Mean	32.775	30.05

Variance	64.11583333	186.55
t Stat	0.845978592	
P(T<=t) one-tail	0.22985184	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.459703681	
t Critical two-tail	3.182446305	

	2.45µg IIa & 4.10µg ATIII	*0.5µg SPD*
Mean	32.775	35.825
Variance	64.11583333	25.2225
t Stat	-0.471247413	
P(T<=t) one-tail	0.334807562	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.669615124	
t Critical two-tail	3.182446305	

Gamma Complex P Values

Gamma [IIa-ATIIII]

	2.45µg IIa & 4.10µg ATIII	*1.0µg Heparin*
Mean	15.575	30.725
Variance	16.71583333	11.7425
t Stat	-4.058561511	
P(T<=t) one-tail	0.013480582	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.026961164	
t Critical two-tail	3.182446305	

	2.45µg IIa & 4.10µg ATIII	*1.0µg PS*
Mean	15.575	3.035
Variance	16.71583333	1.184966667
t Stat	4.894387654	
P(T<=t) one-tail	0.008159173	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.016318346	
t Critical two-tail	3.182446305	

	2.45µg IIa & 4.10µg ATIII	*0.07µg SPM*
Mean	15.575	8.0575
Variance	16.71583333	1.781091667
t Stat	5.377275799	
P(T<=t) one-tail	0.006297419	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.012594838	
t Critical two-tail	3.182446305	

	2.45µg IIa & 4.10µg ATIII	*0.7µg SPM*
Mean	15.575	8.49
Variance	16.71583333	2.514466667
t Stat	4.127209753	
P(T<=t) one-tail	0.012898409	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.025796817	
t Critical two-tail	3.182446305	

	2.45µg IIa & 4.10µg ATIII	*0.05µg SPD*
Mean	15.575	9.43

Variance	16.71583333	3.775266667
t Stat	4.861937628	
P(T<=t) one-tail	0.00830867	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.016617341	
t Critical two-tail	3.182446305	

	2.45µg IIa & 4.10µg ATIII	*0.5µg SPD*
Mean	15.575	4.595
Variance	16.71583333	8.063366667
t Stat	11.41923837	
P(T<=t) one-tail	0.000720556	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.001441112	
t Critical two-tail	3.182446305	

IIa Beta complex goes down with Heparin. IIa Gamma complex goes up when in the presence of Heparin, consistently. IIa Alpha complex stays near a similar value when in the presence of Heparin.

This means that Heparin promotes the overall degradation of IIa-Alpha ATIII to IIa- Gamma ATIII.
PS:
There is a noticeable large increase in the Alpha Complex when in the presence of PS. For Beta there is a decrease when in the presence of PS. The Gamma IIa complex experiences a large decrease when in the presence of PS.
0.07 SPM:
IIa Alpha shows a large increase in the presence of 0.07SPM. IIa Beta shows a slight increase. However, IIa-Gamma, shows consistent decrease when in the presence of 0.07SPM.
0.7 SPM
IIa Alpha shows a large increase when in the presence of 0.7 SPM. IIa Beta maintains its complex when in the presence of 0.7SPM. IIA Gamma shows a large decrease when in the presence of 0.7 SPM.
SPM's by in large act similarly to PS.
0.05 SPD
IIA alpha all show a large increase when in presence of 0.05SPD. IIa beta maintains complex in comparison to control when with 0.05 SPD. The gamma IIA complex shows a large decrease, consistently.
0.5SPD
IIA alpha shows a large increase in the presence of 0.5 SPD. IIa Beta maintains the complex mostly. IIa Gamma shows a large decrease.

Overall, the SPD also acts like SPM, which acts more like PS.

Brief Analysis:
Protamine Sulfate appears to promote the creation of the Alpha [IIa-ATIII] Complex while nearly eliminating the Beta [IIa-ATIII] Complex. Heparin does the opposite. SPM and SPD appear to act more similarly to Protamine Sulfate. No IIa was detected; perhaps it was all used in the complex formation or left in the loading dye.

Interpreting the Results

Overall, it would appear that Spermine and Spermidine act more like Protamine Sulfate than Heparin, inhibiting complex formation. However, Spermine and Spermidine do not completely block out the complexes in any experiment but significantly lessen them when compared with Heparin.

In the IXa experiments, Protamine Sulfate completely blocked the formation of the [IXa- ATIII] band. Spermine and Spermidine both inhibited the complex but did not completely eliminate it.

In the Xa experiments, it is harder to determine how Spermine and Spermidine act. However, when observing the P Values as well as the photographs and the coorosponding data, we can make fairly accurate determinations. The p-values are the best in the primary alpha complex while the primary beta has low but not super desireable values. The tertiary alpha and beta values are higher but this is expected due the the degradation required to move from complex to complex in the blood cascading system. When observing the photographs, we see that Heparin has dark primary alpha and beta bands while protamine sulfate has lower bands. Spermine and Spermidine have primary alpha and beta bands that are darker than protamine sulfate and either equivalent or under that of heparin. The most noticeably change is the behavior in the tertiary complexes. We see Protamine Sulfate form darker tertiary alpha and beta bands than Heparin. Spermine and Spermidine form tertiary bands but their formation appears to resemble that of protamine sulfate. Human Factor Xa may benefit from more experiments but there will see be differences in values because of how quickly complex degradation can happen.

In the XIa experiment, Spermine and Spermidine appear to completely block the [XIa-$(ATIII)_2$] secondary complex while there is a huge decrease in the [XIa-ATIII] complex

when compared with heparin. Heparin has a small [XIa-$(ATIII)_2$] secondary complex formation and a significant [XIa-ATIII] complex while Protamine Sulfate does not appear to form any secondary complex. Therefore, we see that Spermine and Spermidine are acting more like Protamine Sulfate.

In the IIa experiments, we see that Spermine and Spermidine increase the formation of the alpha and beta [IIa-ATIII] complexes but there is a significant decrease in the gamma [IIa-ATIII] complex. Heparin, however, has small amounts of the alpha and beta complexes and a large amount of the gamma. This is due to Heparin promoting the degeneration from alpha, to beta, and then to gamma. Protamine Sulfate appears to form large alpha and beta complexes and a small gamma complex in the experiments. Therefore, it is seen that Spermine and Spermidine act most like Protamine Sulfate when in the presence of IIa.

Therefore, we see that Spermine and Spermidine act more like Protamine Sulfate and inhibit complex formation. Since Heparin is defined as a coagulant, based on these experiments, it may be acceptable to classify Spermine and Spermidine as anti-coagulants. However, it may be more acceptable to note these two polyamines as complex inhibitors.

What I Have Gained

My learning style enables me to thrive in laboratory environments. I enjoy getting to be hands and learn from what I see and what I do since it is all very concrete. I actually have not taken my biochemistry course yet, that is my senior year science class. I believe it would be easy to say that I will have a slight leg up on my future classmates, at least in the laboratory portion.

I have learned how to write proper scientific reports and how to determine if my results are accurate. This can be seen in this honors project, my use of p-values and the interpretation of such values, as well as my other lab reports. I have learned what is expected in a publication, since it is my intention to have this work published. I have also learned a lot about collaboration. I have had to collaborate between myself and my mentor (Dr. Brecher), those who have assisted me, as well the future publishers since these experiments do have to meet their standards.

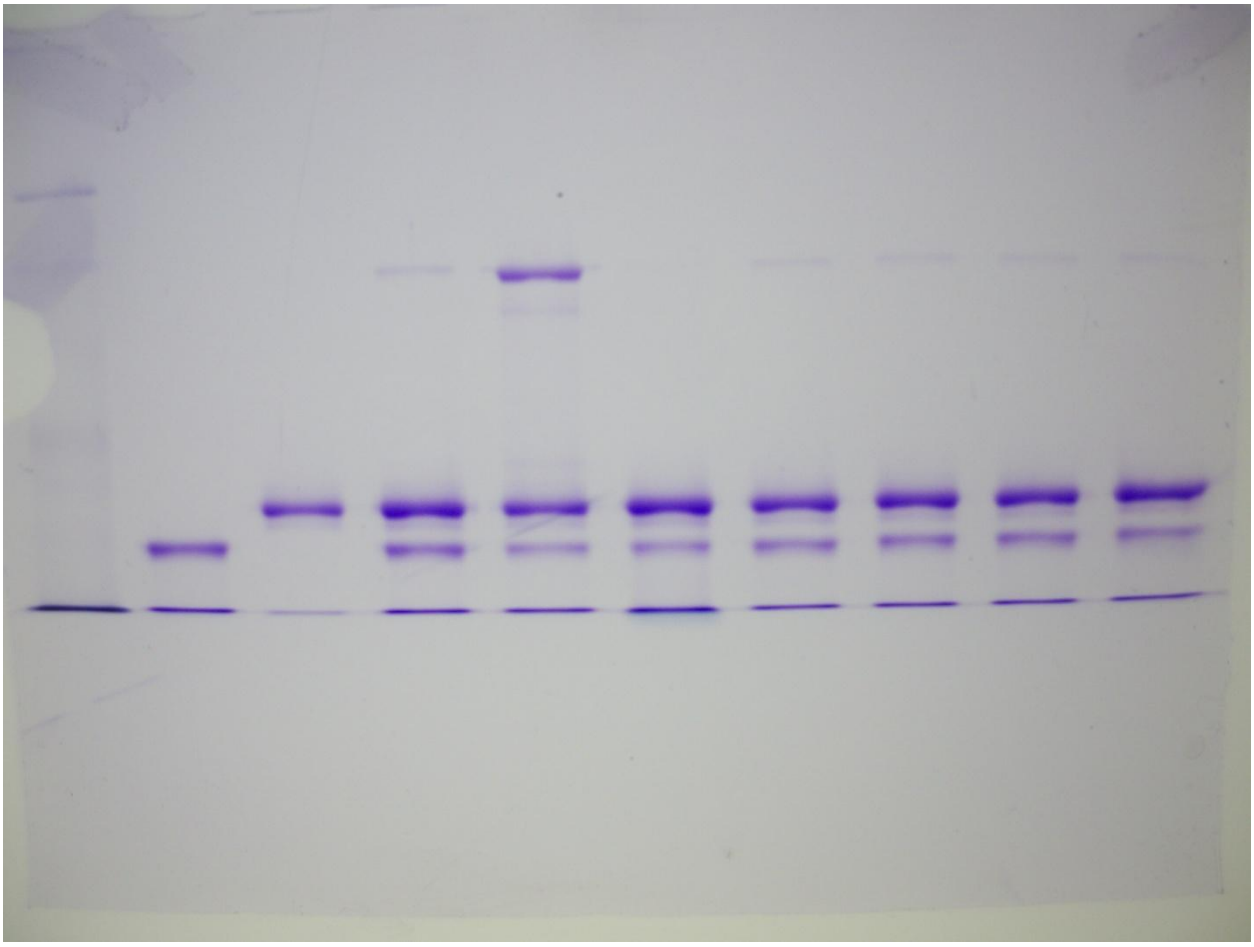
This academic research has allowed me to grow as a student and young adult as well. The research, while time consuming, has forced me to maintain adequate time management but taught me about where my strengths are and where I can improve. However, I think the most important thing I have gained is enjoyment. I am not required to do research to graduate and it is not required for graduate school. Yet, I have continued to do so because I am good at it and I truly enjoy it.

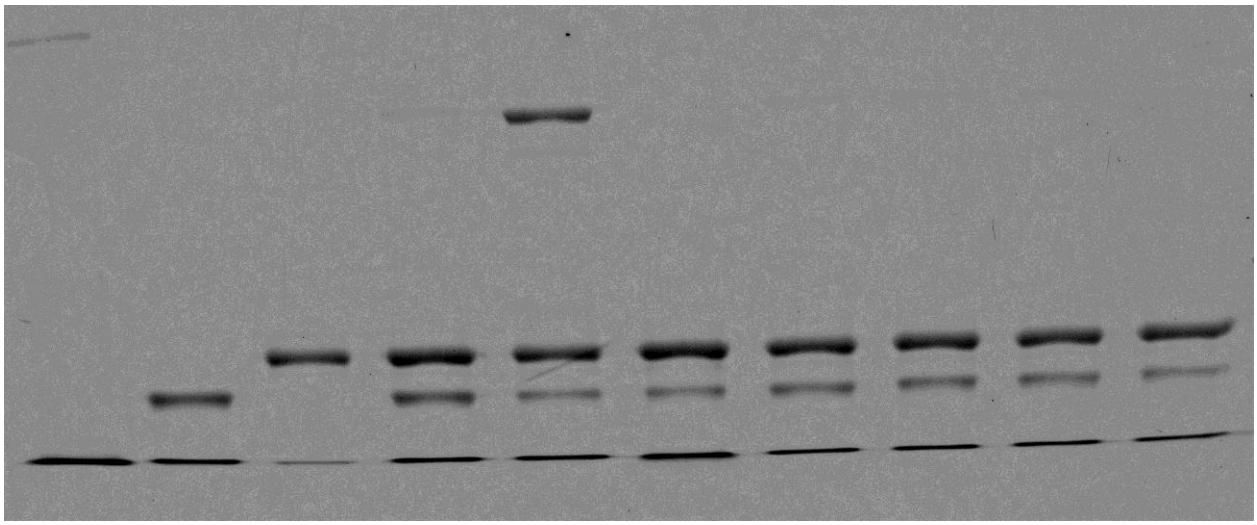
Raw Photographs

Black and White photos are subtracted backgrounds and were used for analysis

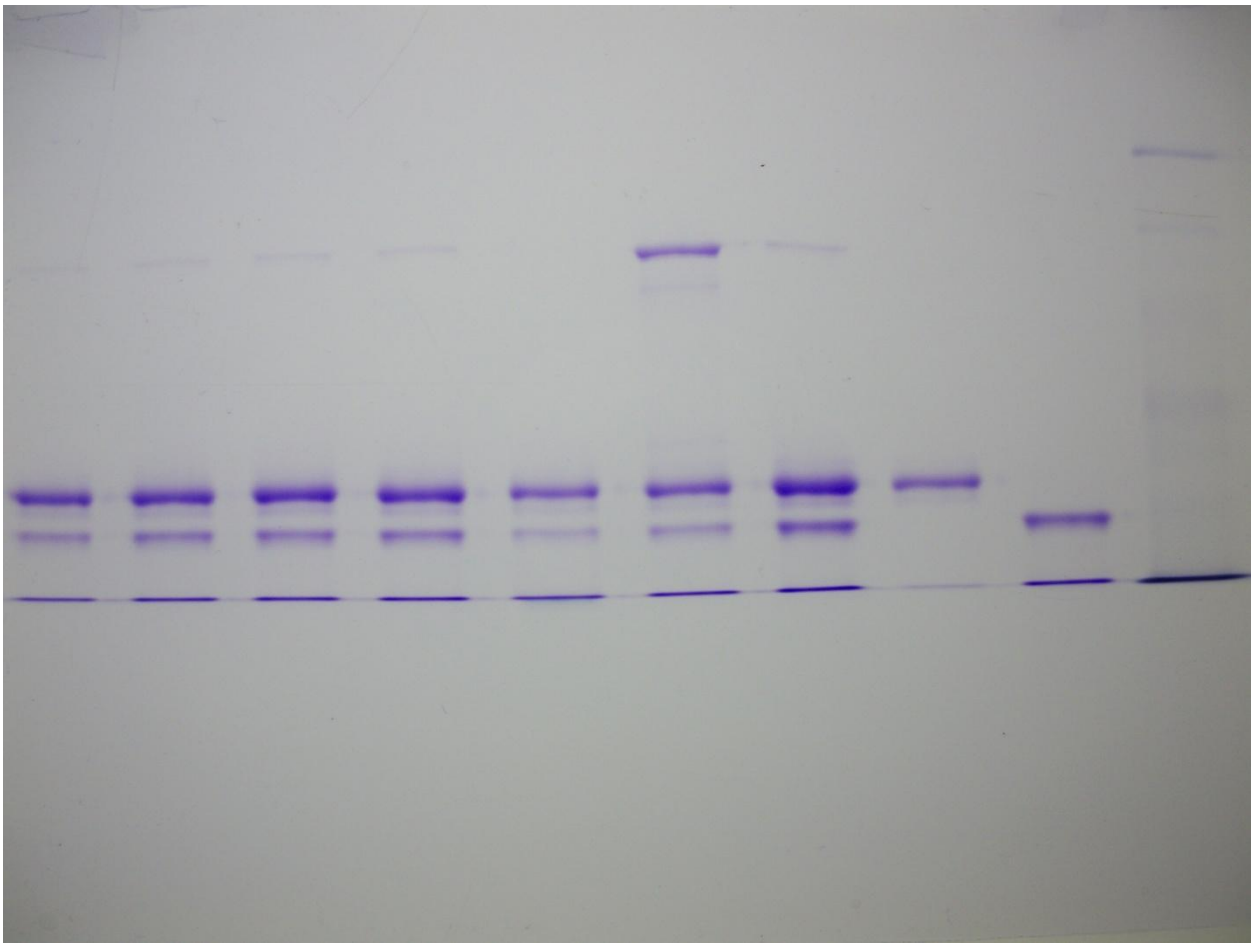
IXa Photos

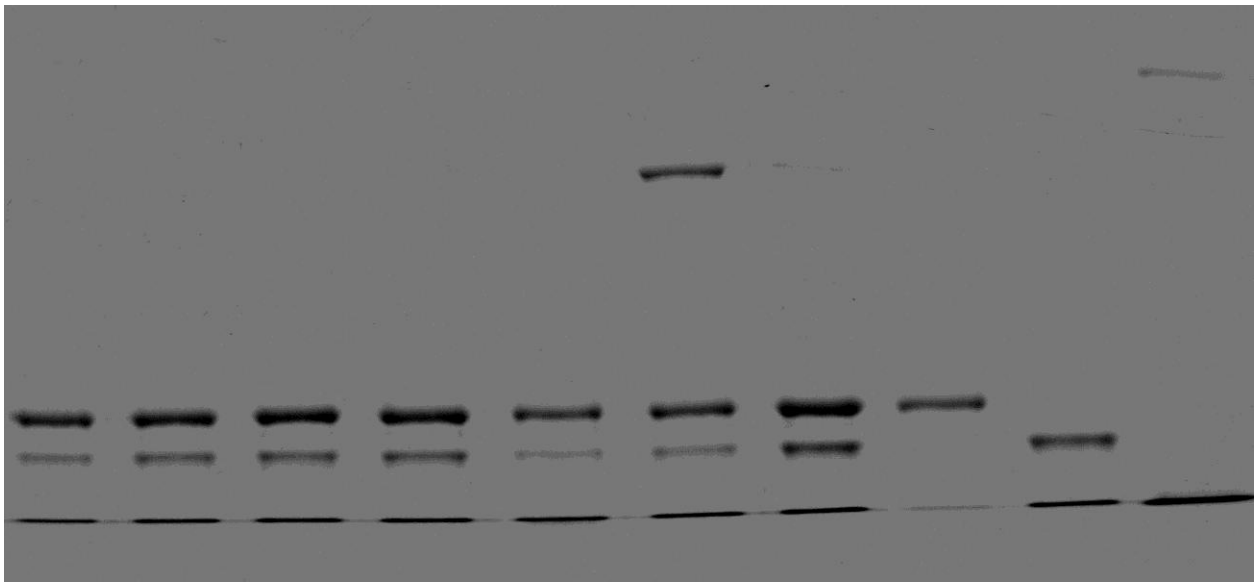
Gel 1:



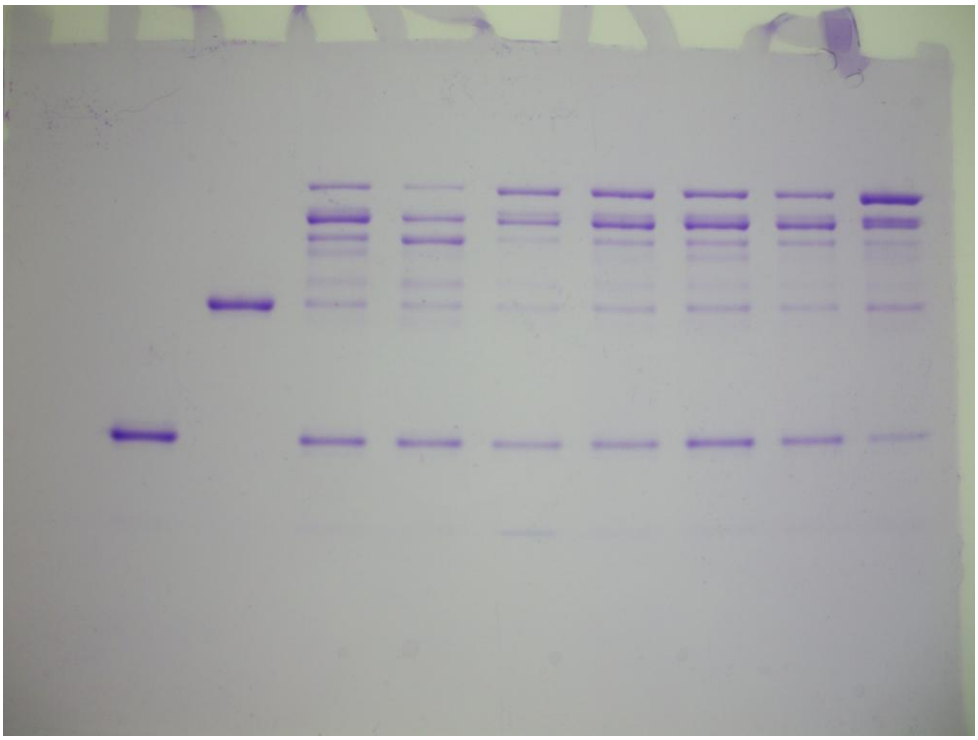


Gel 2:





IIa Gels:
Gel 1:



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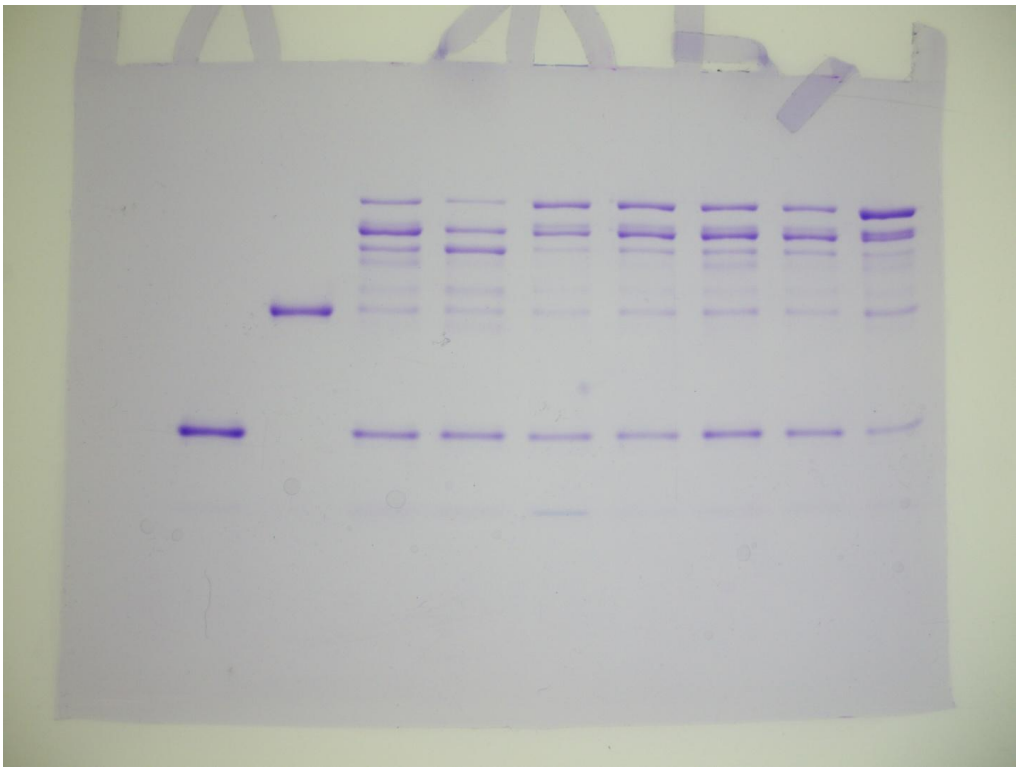
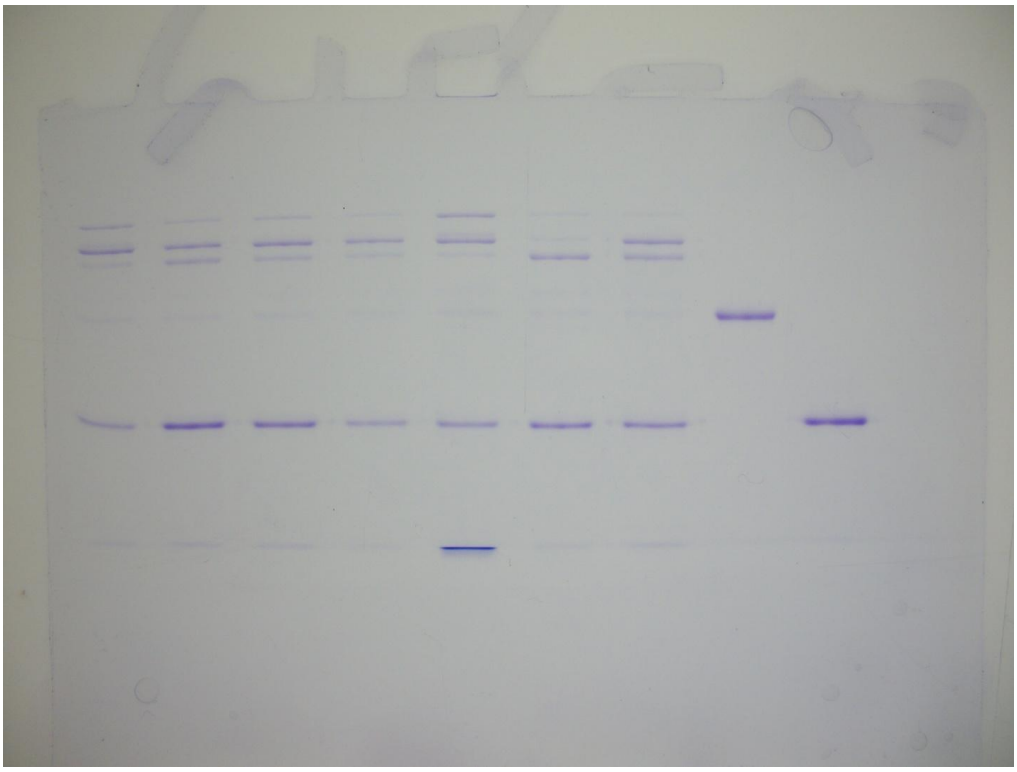
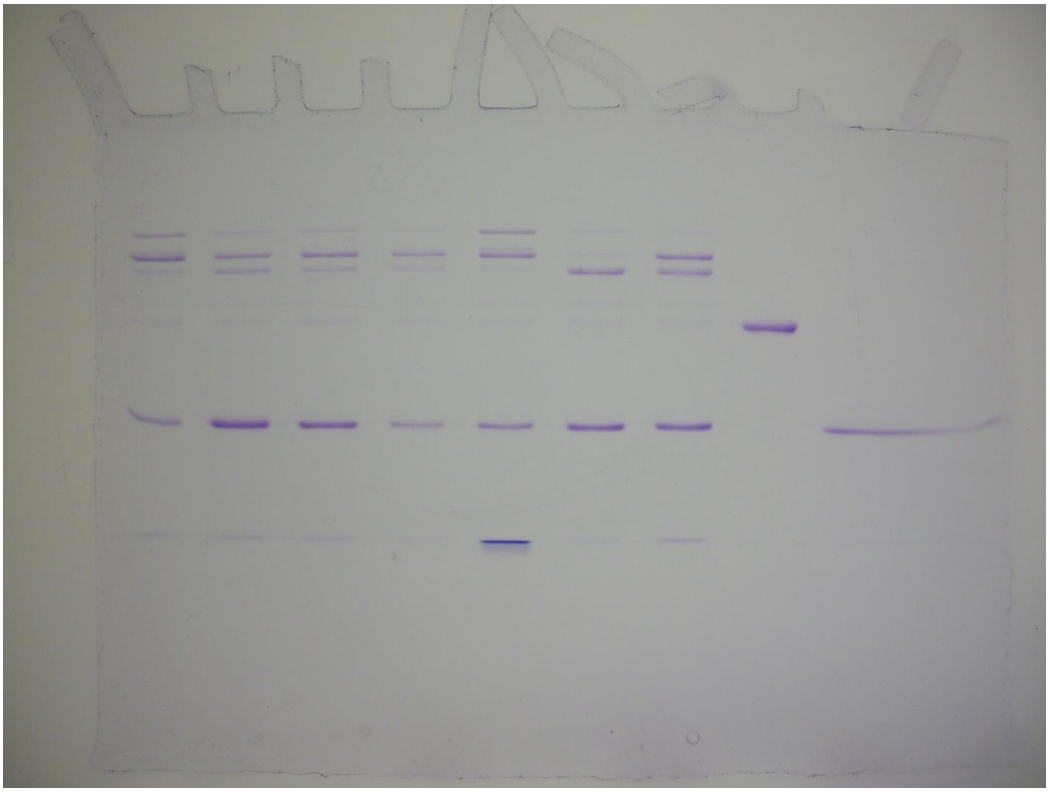


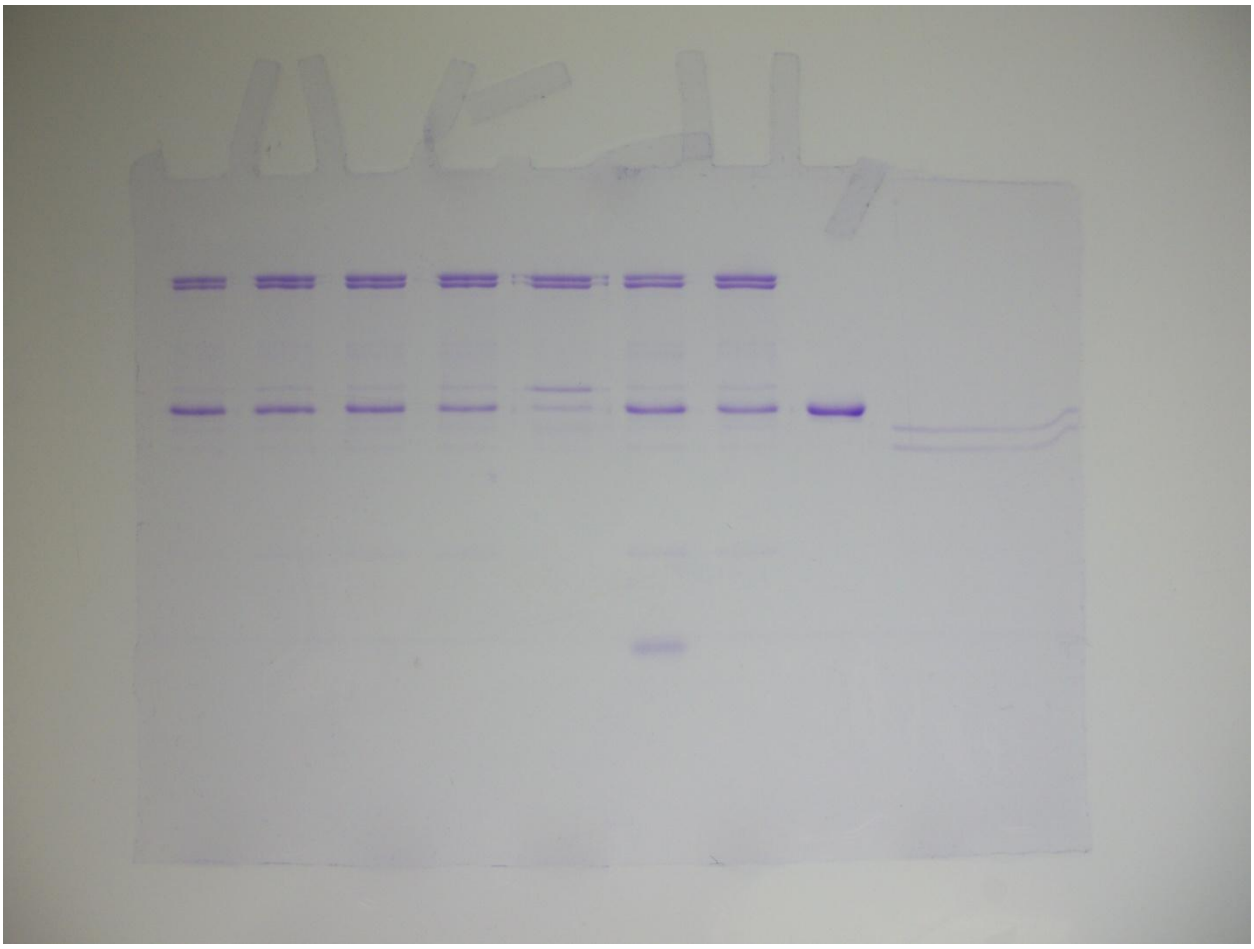
Gel 3:

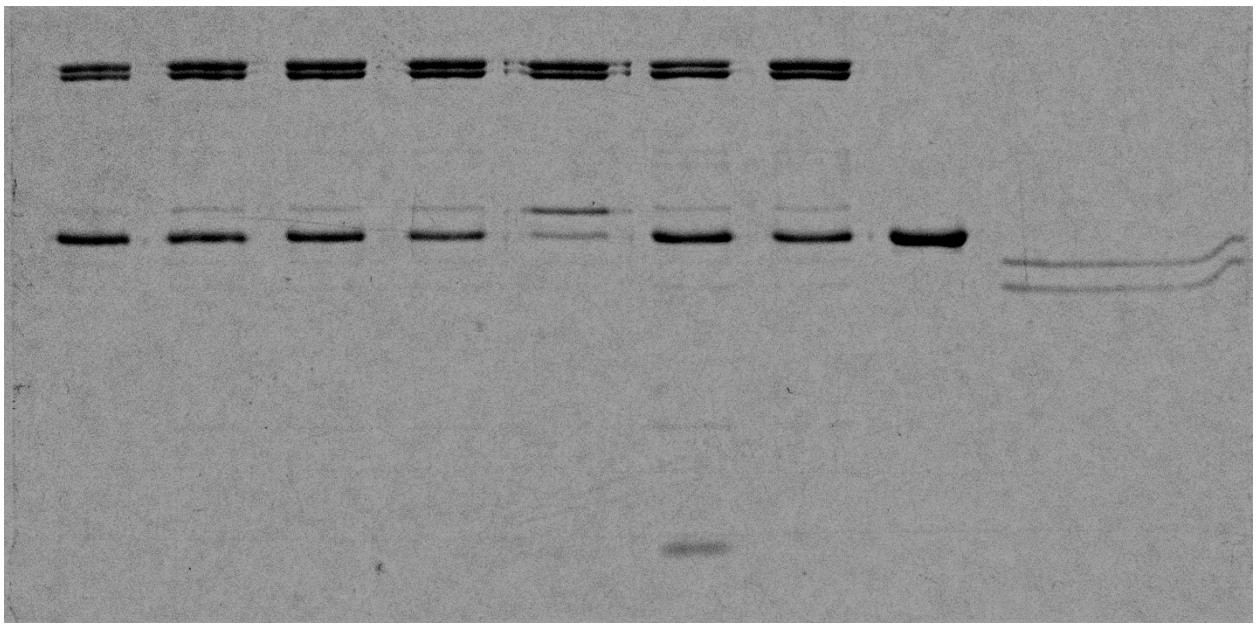


Gel 4:



Xa Gels
Gel 1:





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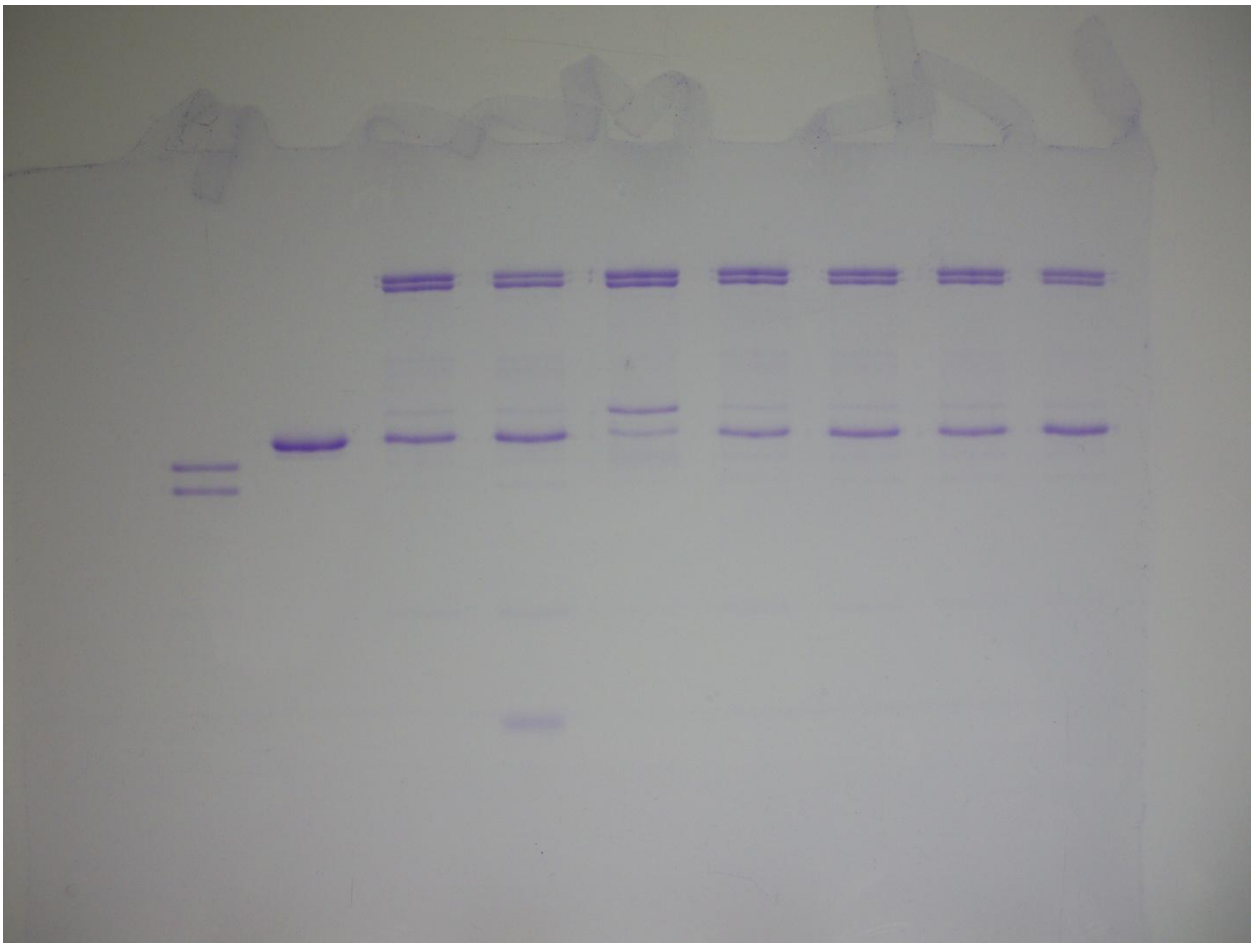

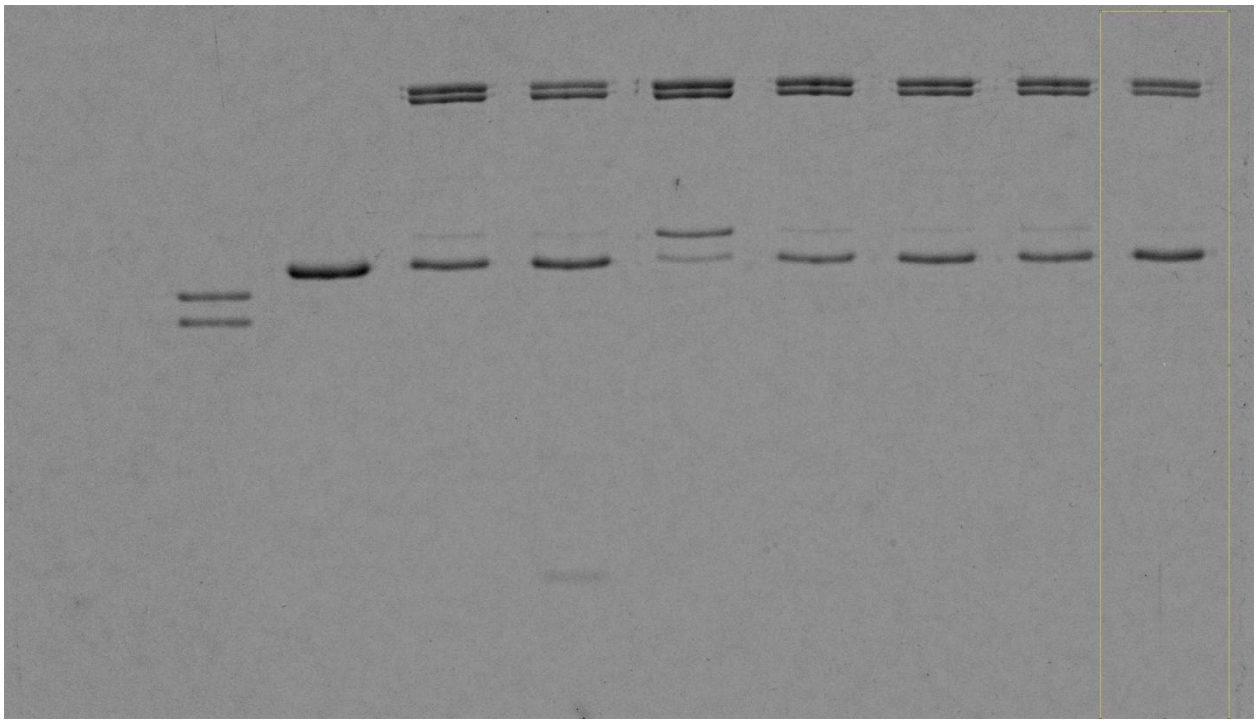

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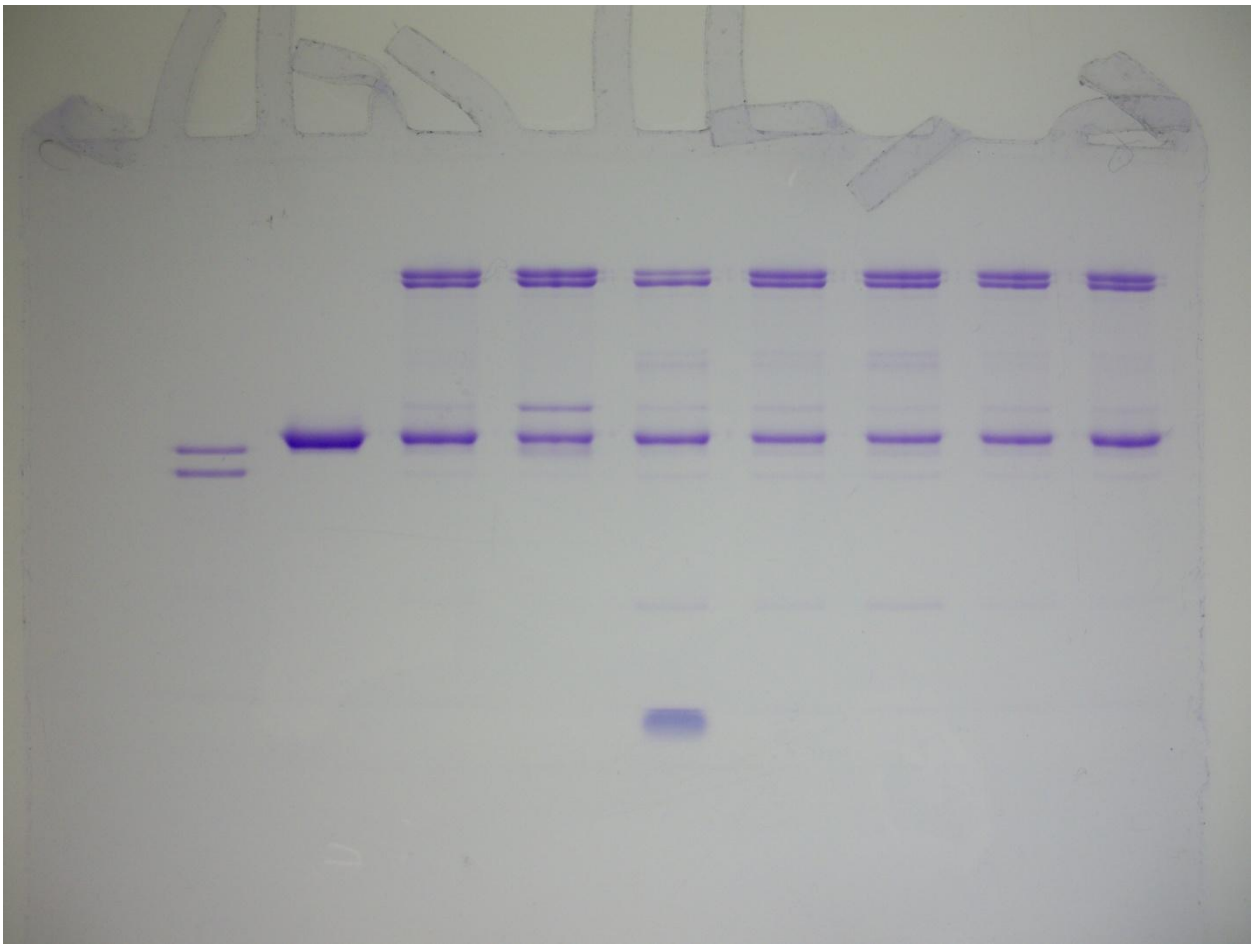
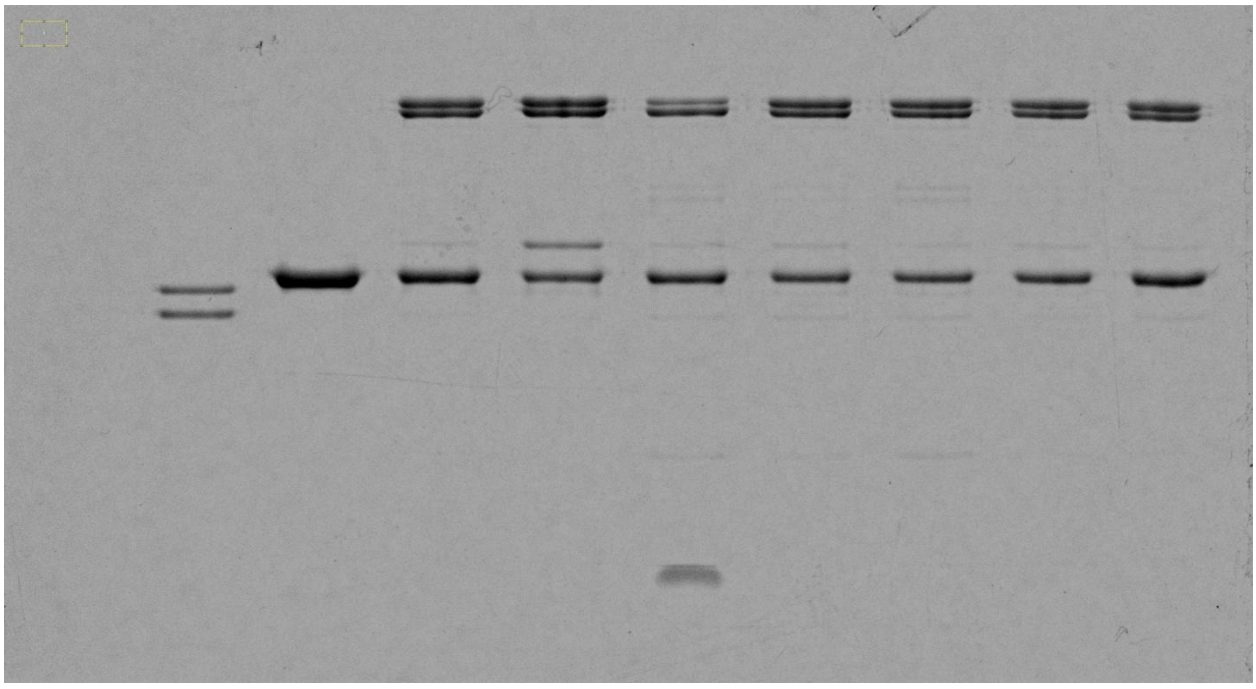
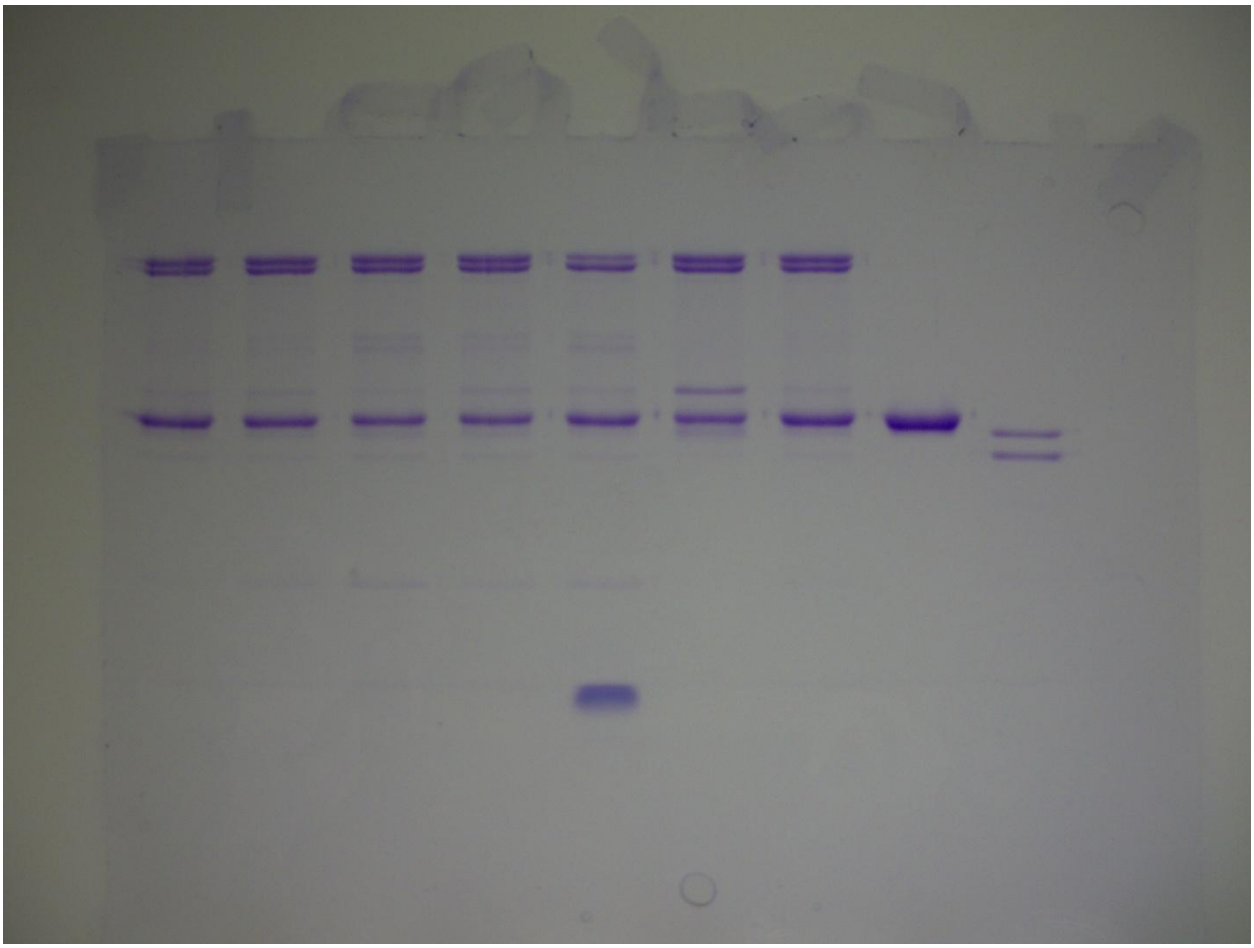




Gel 4:



XIa Gels:
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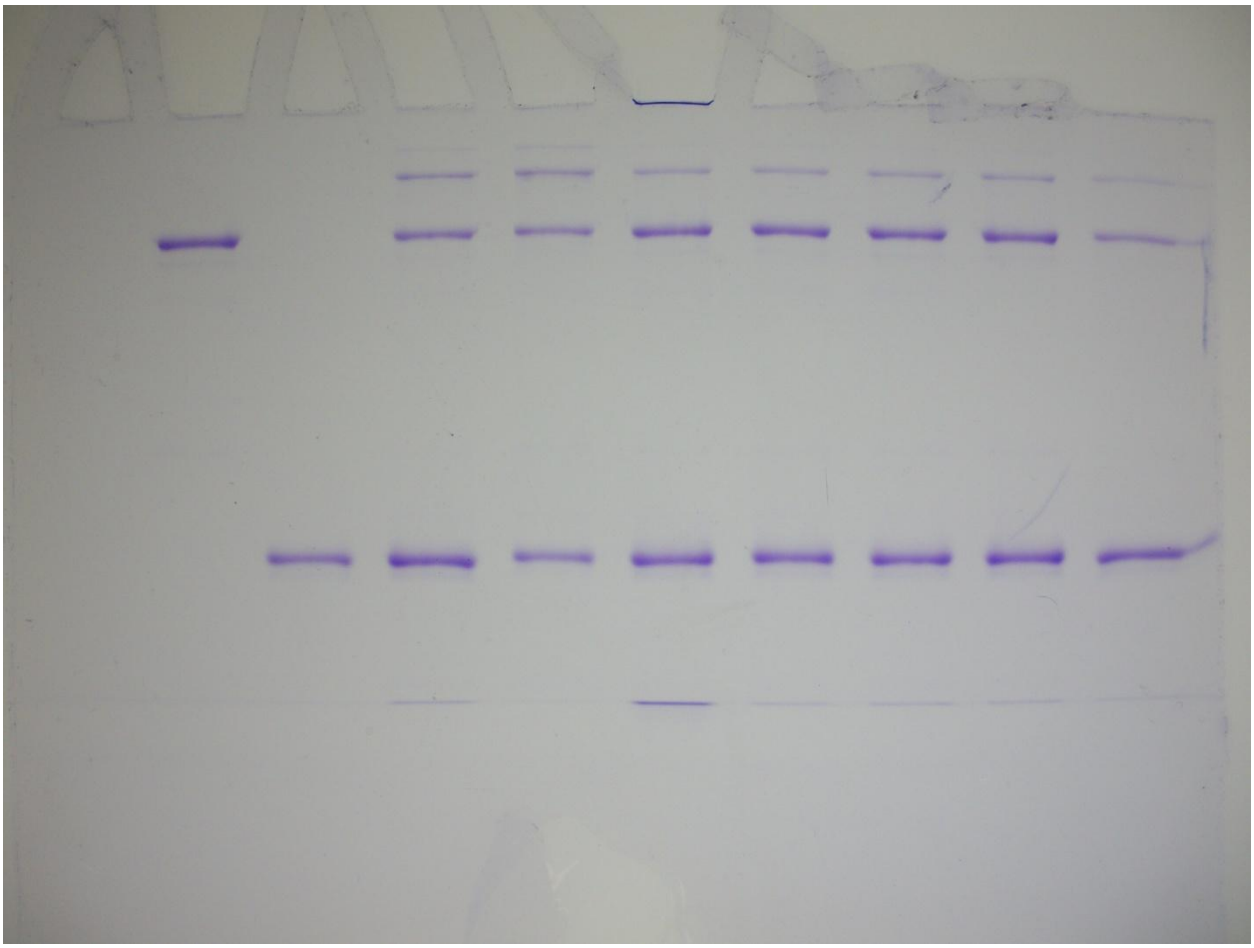
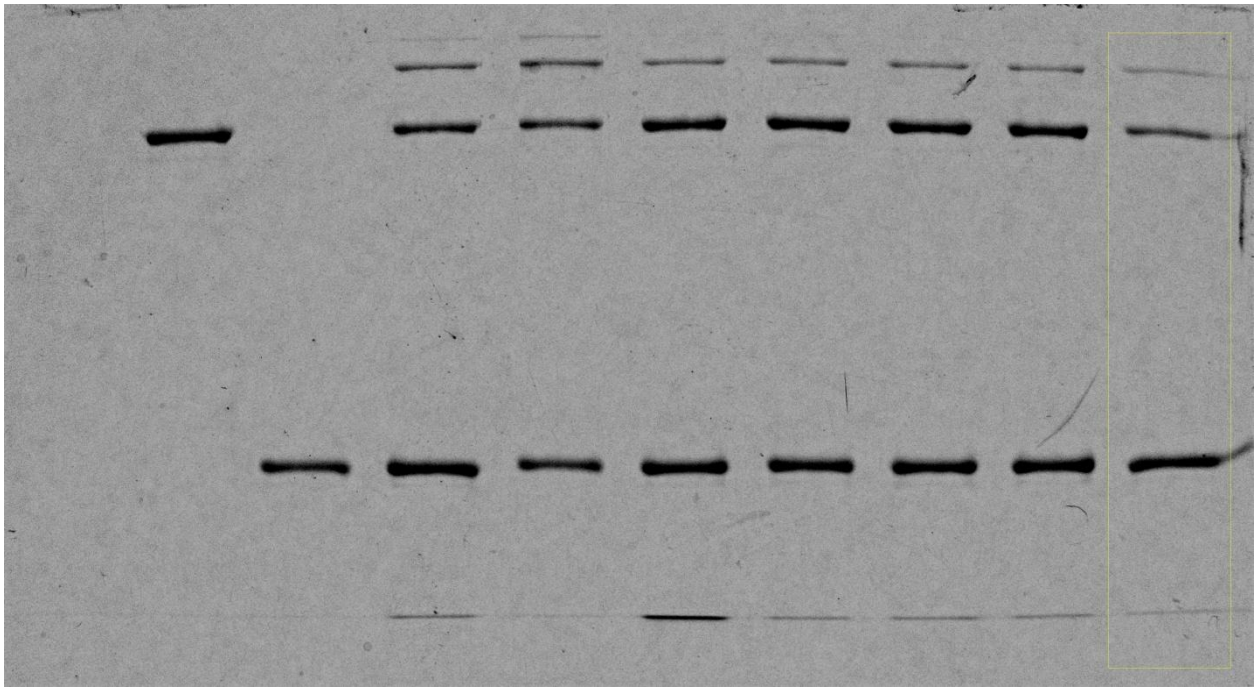
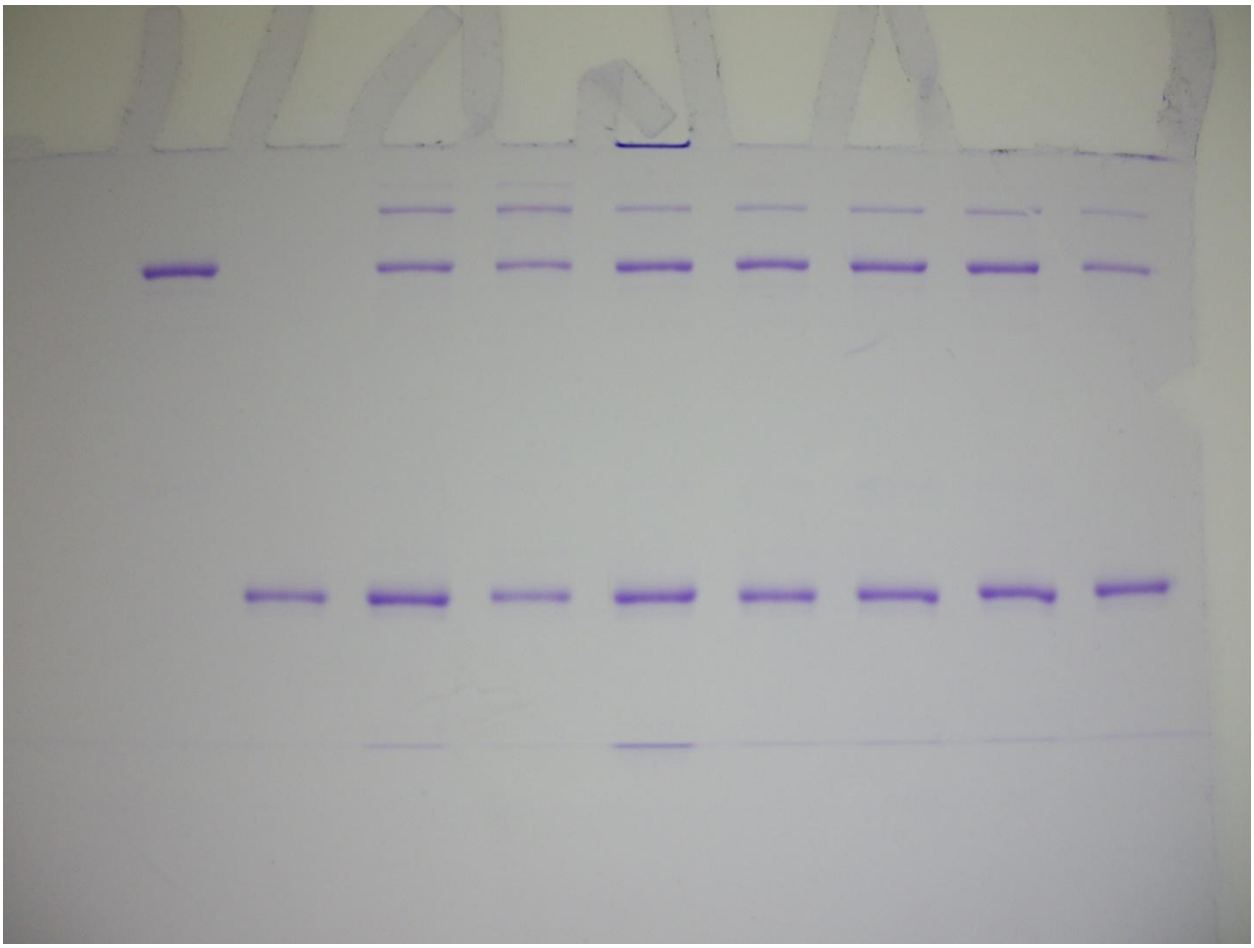
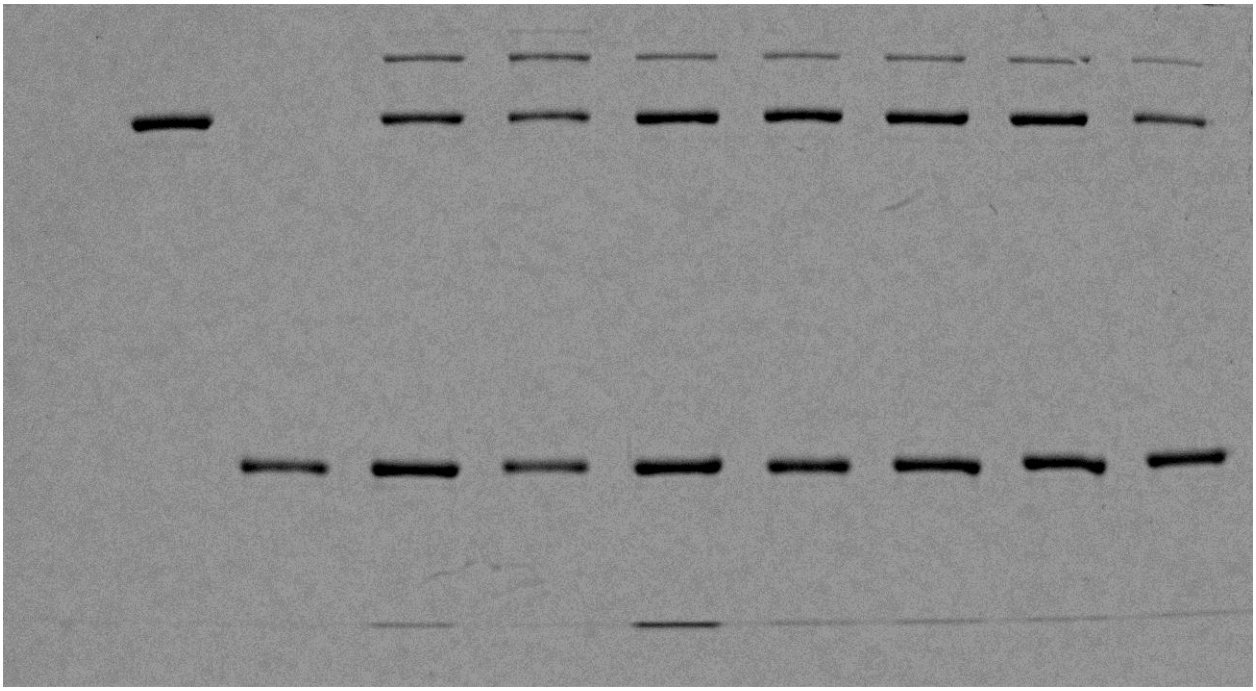




Gel 2:





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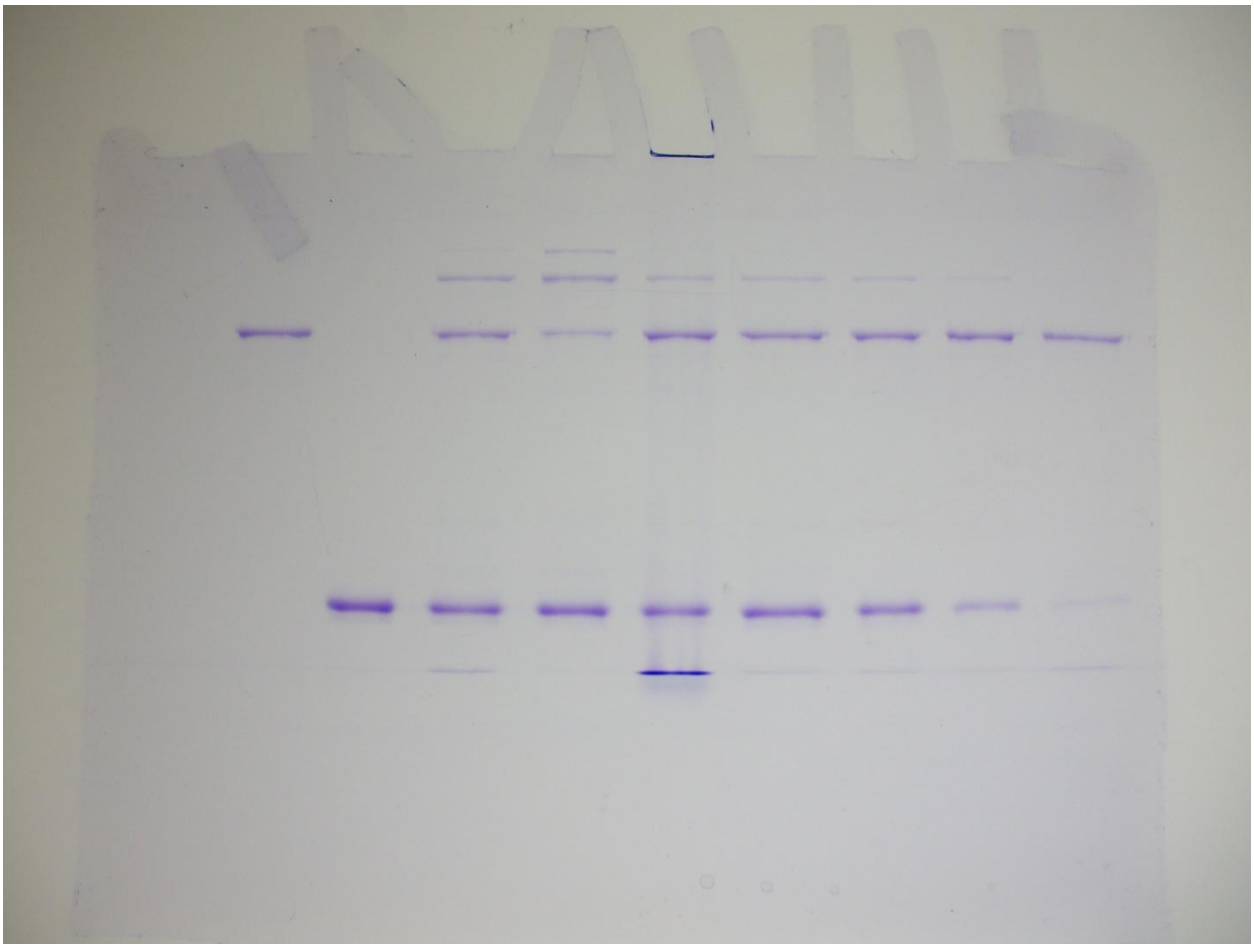
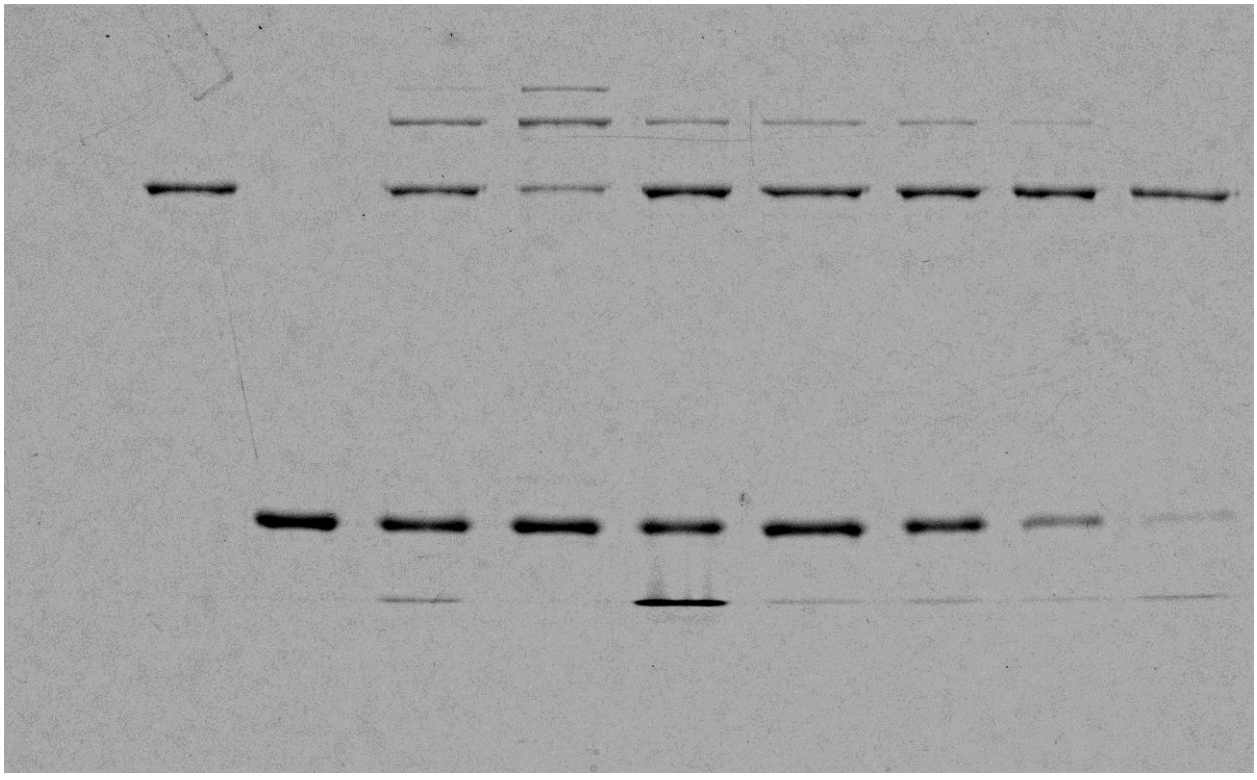
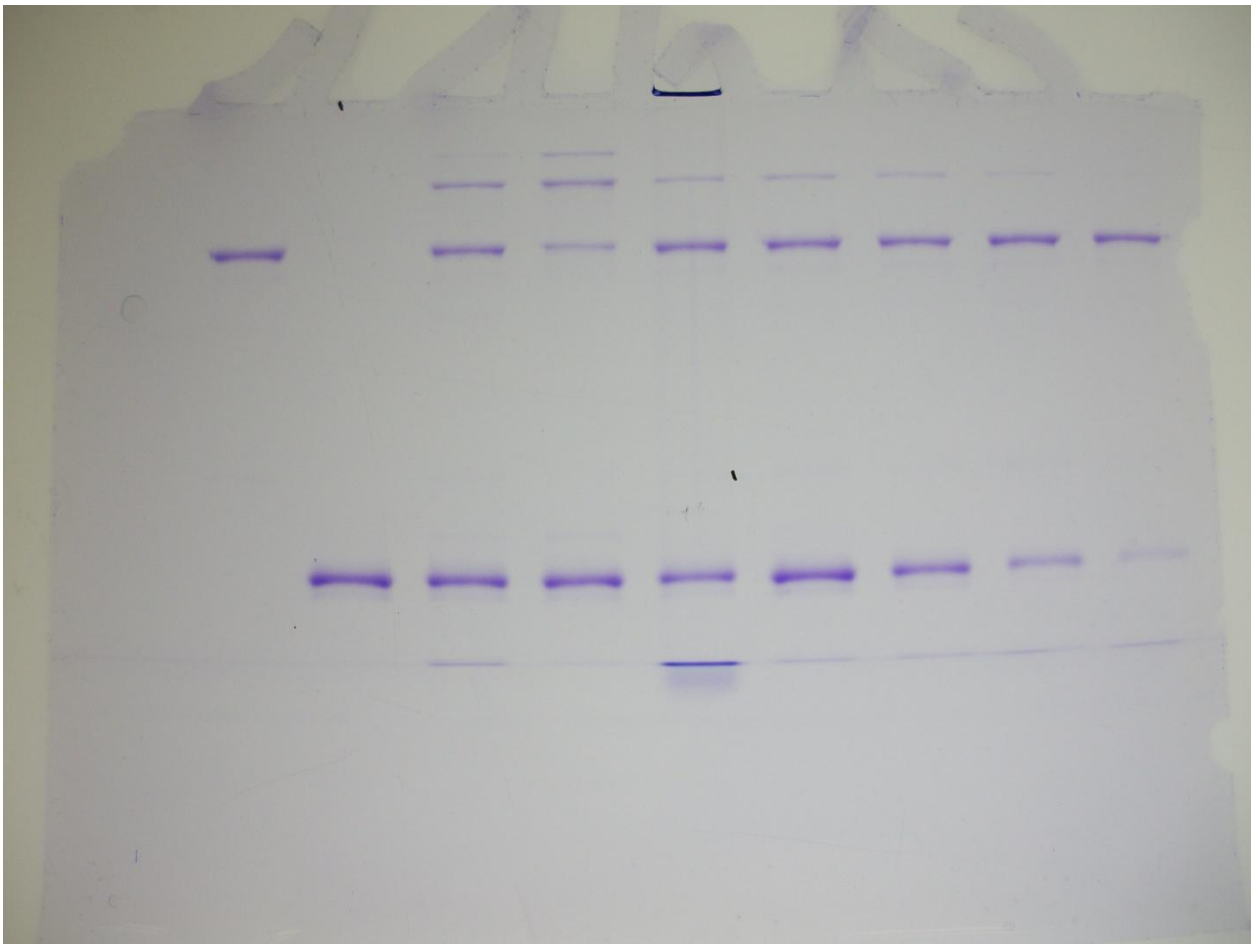
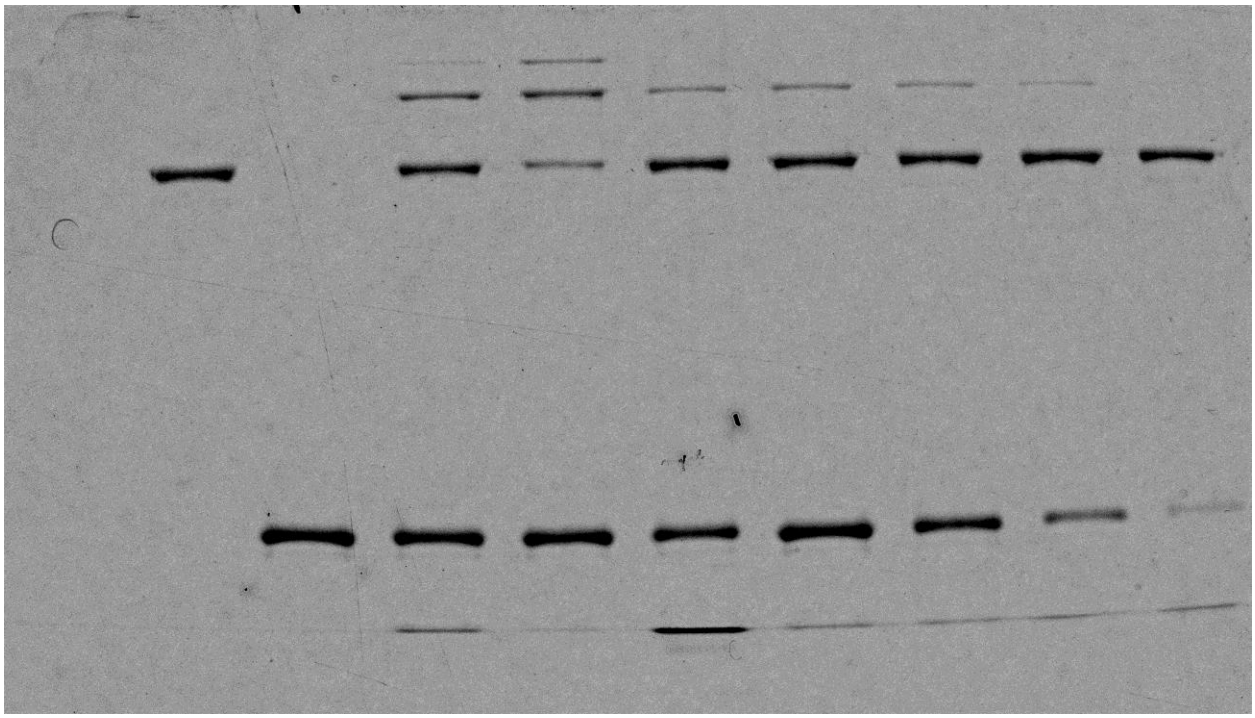




Gel 4:





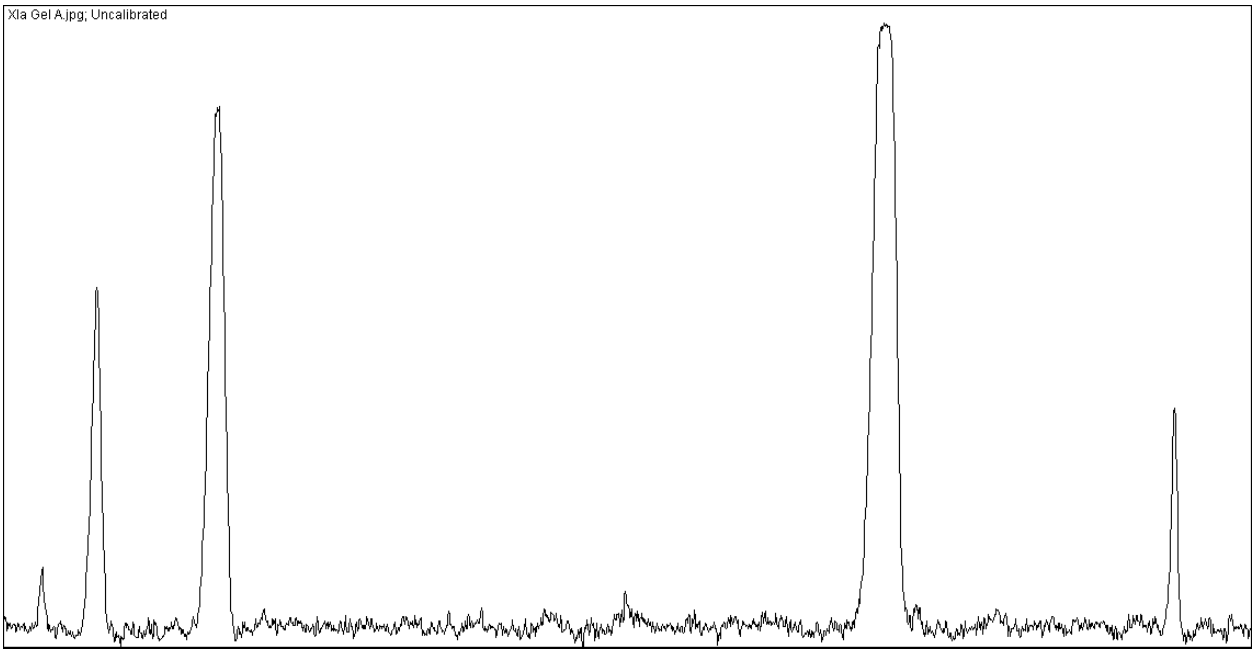
Graphs Used to Determine Percent of Complex Formations

Lane Graph Examples

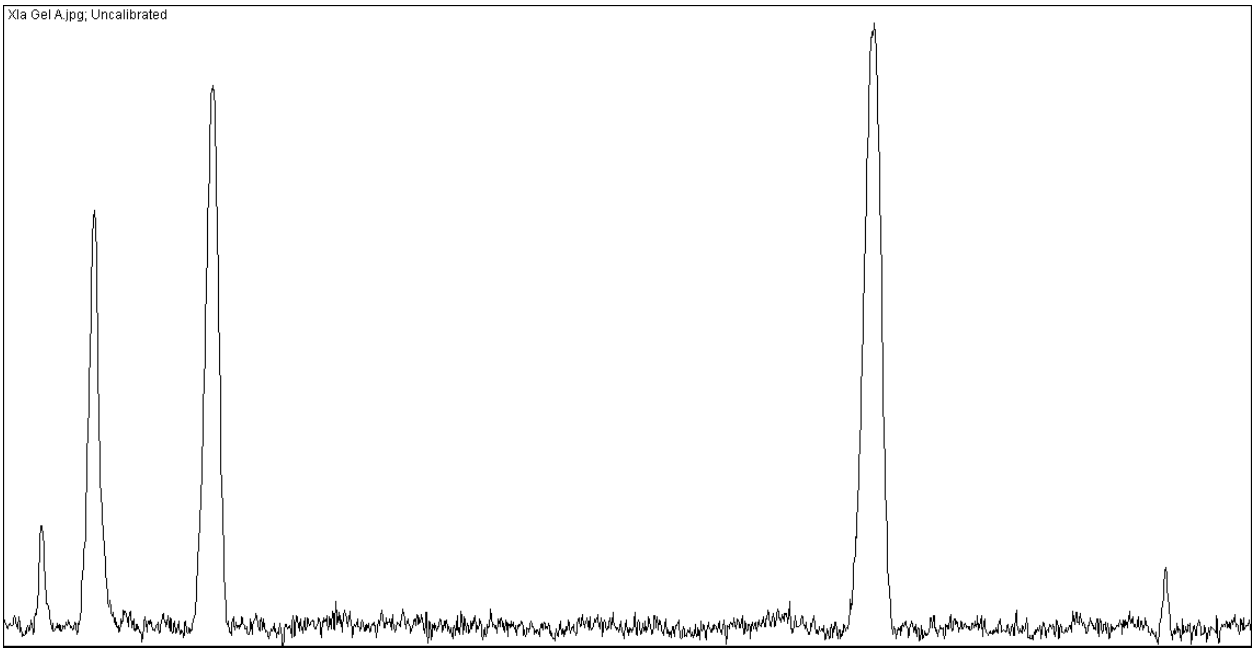
On all graphs, I would use a tool to cut out background noise and separate peaks. I would also double check on the gel to determine where there might be error (black dots, etc.) These errors would also be cut out. Then, the peak percentage would be calculated.

XIa Lane examples

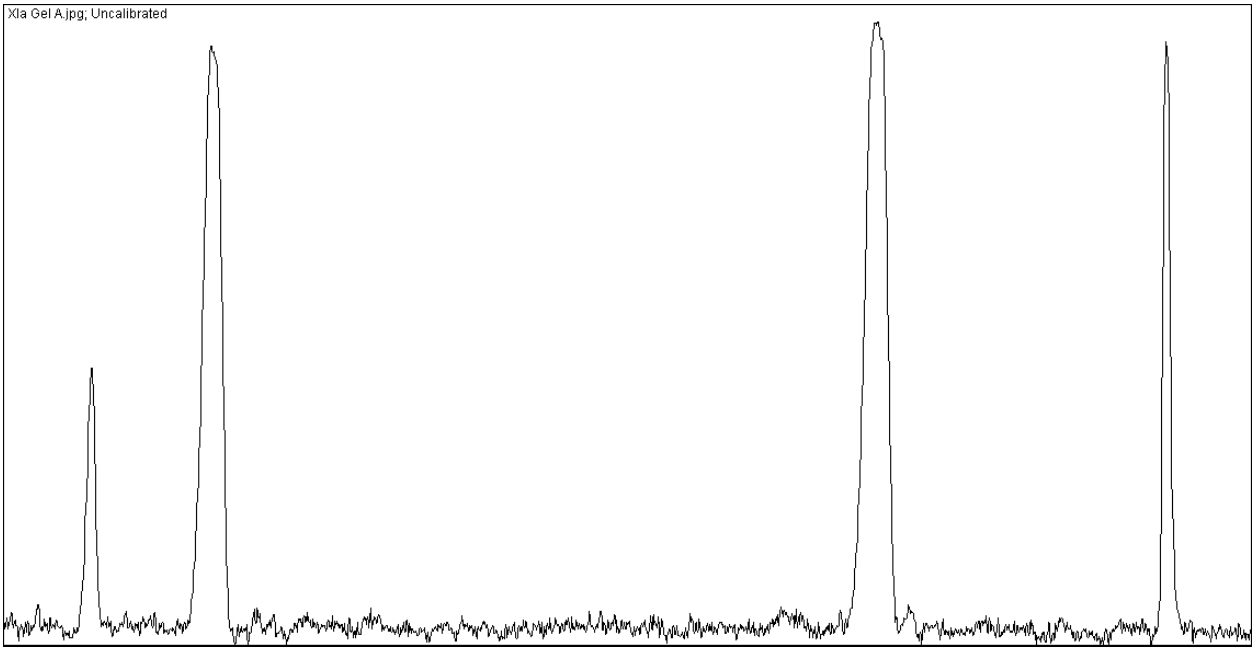
XIa Lane
4



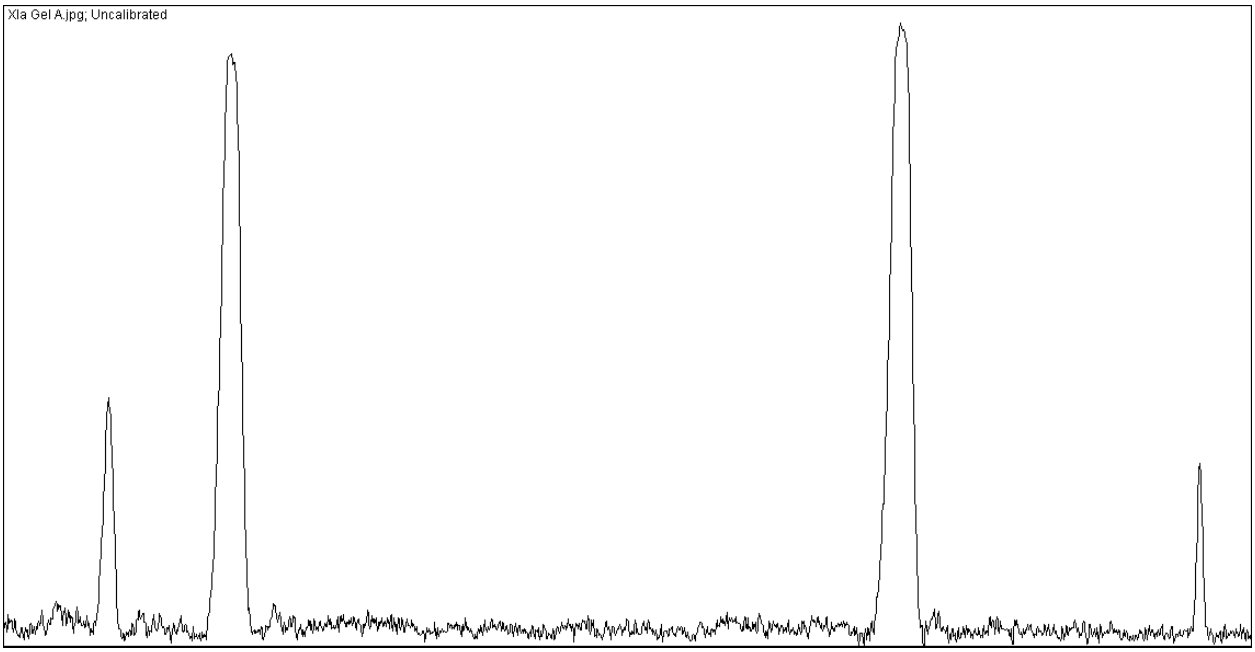
XIa Lane
5



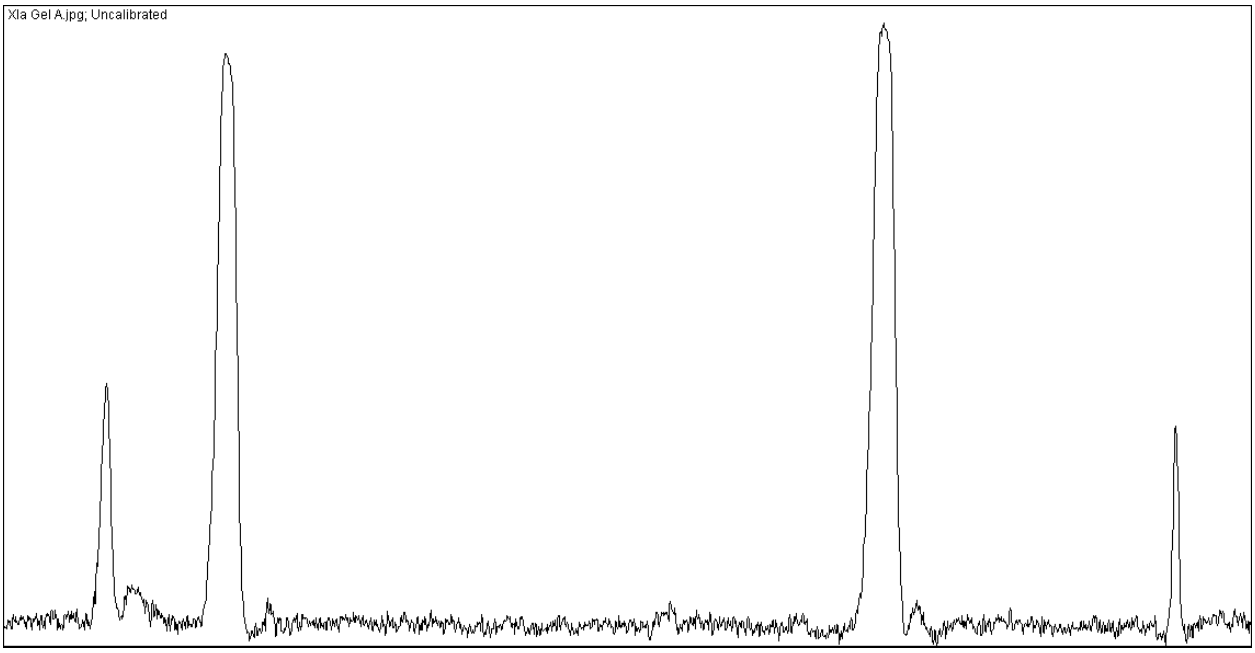
XIa Lane
6



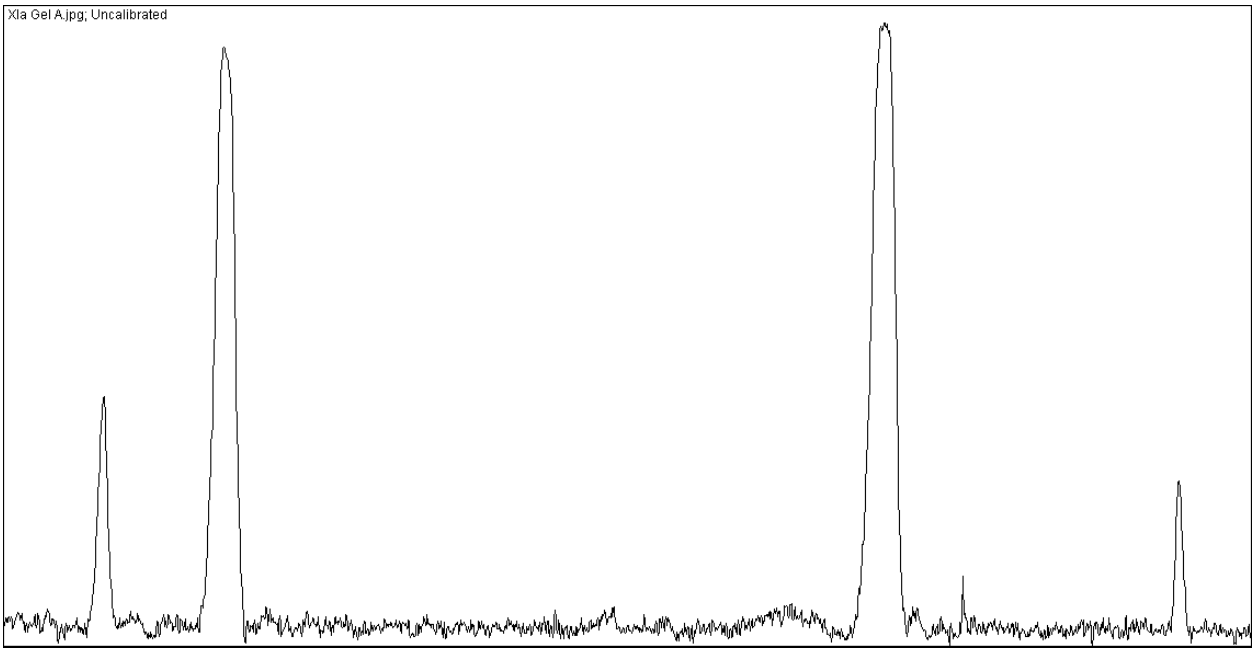
XIa Lane
7



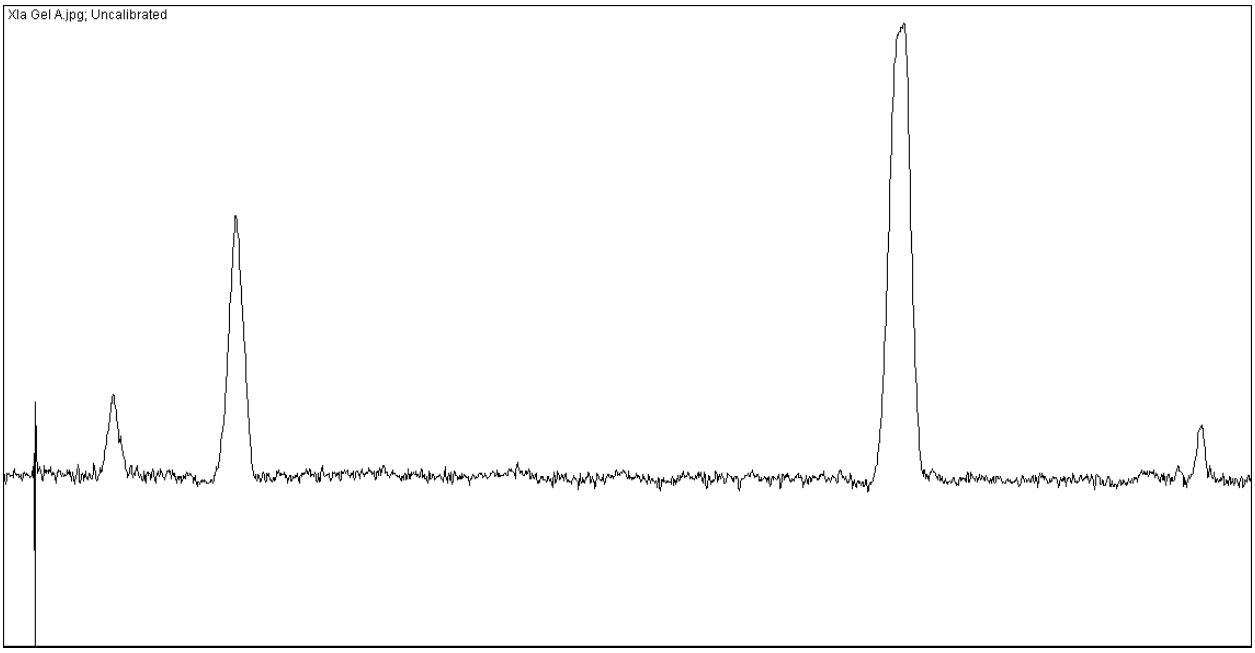
XIa Lane
8



XIa Lane
9

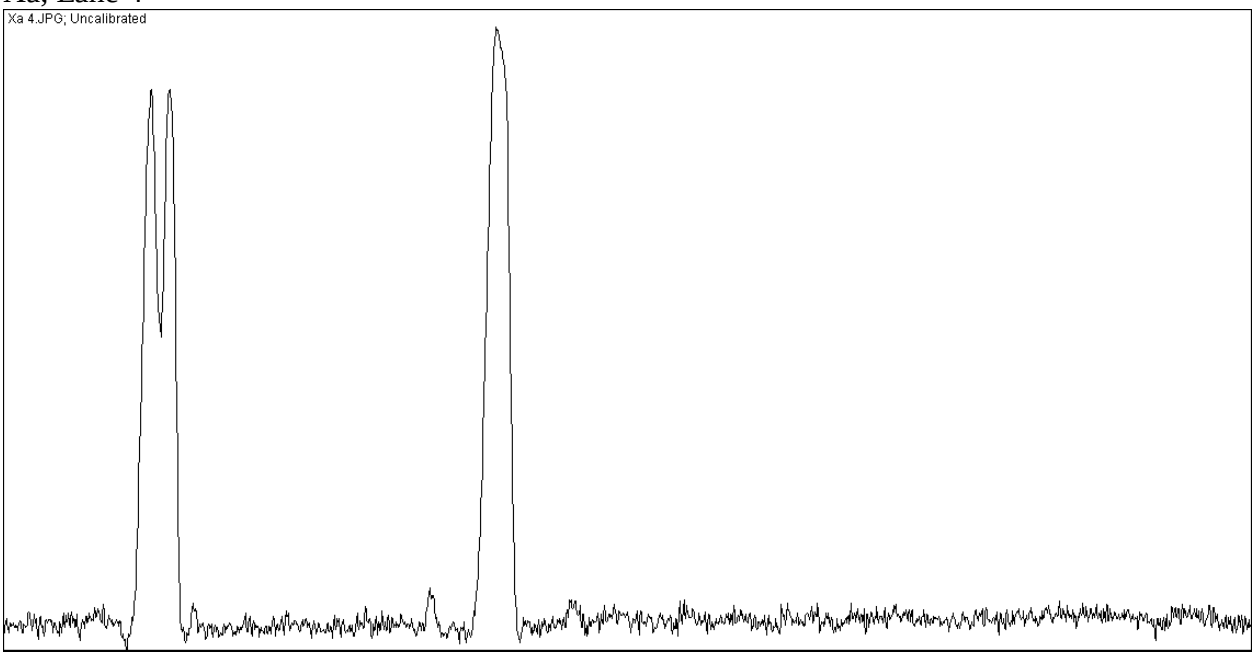


XIa Lane
10

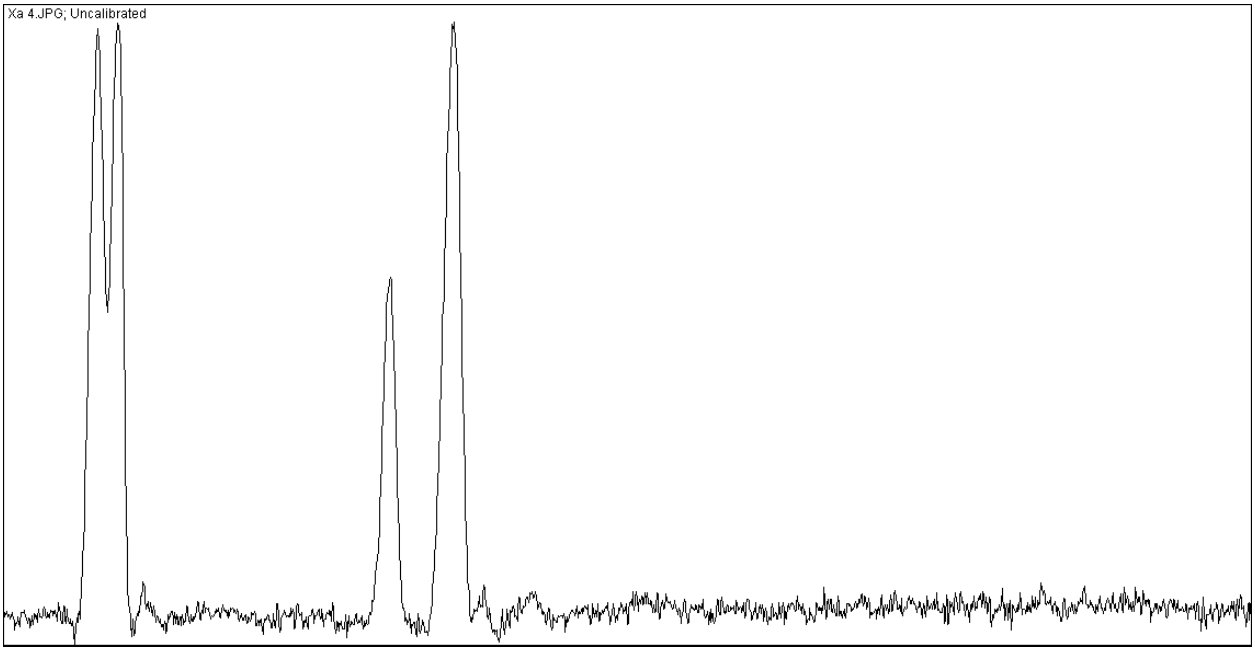


Xa Lane Examples

Xa, Lane 4



Xa, Lane 5



Xa, Lane
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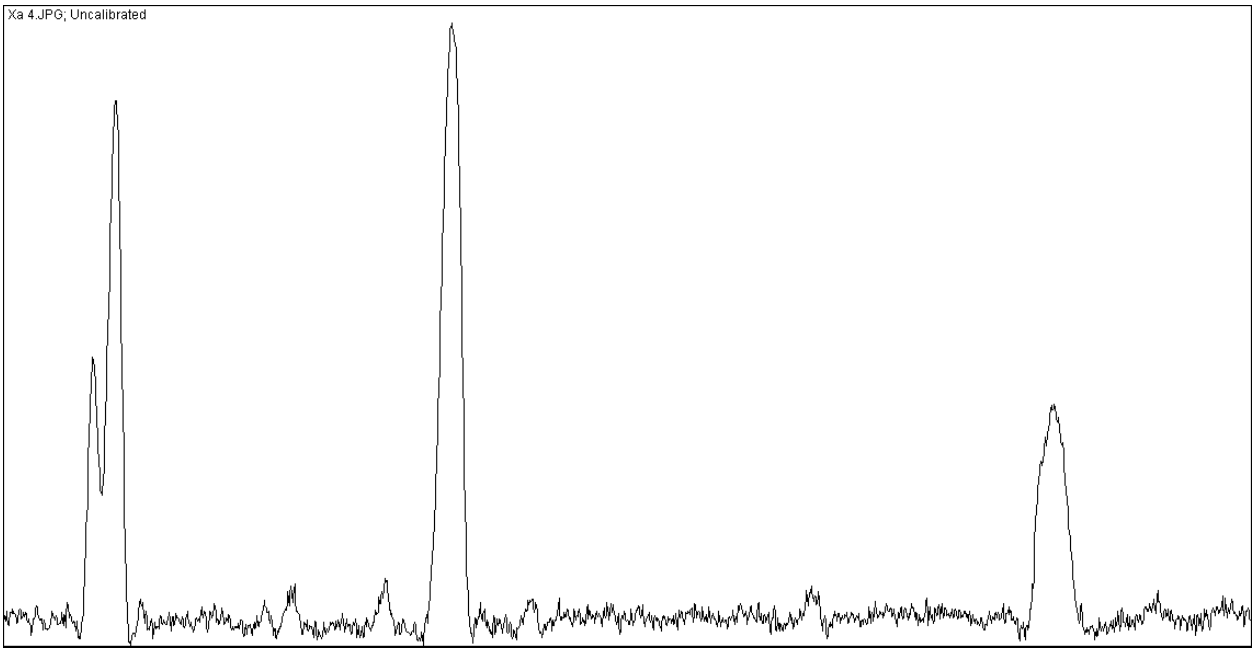

Xa, Lane
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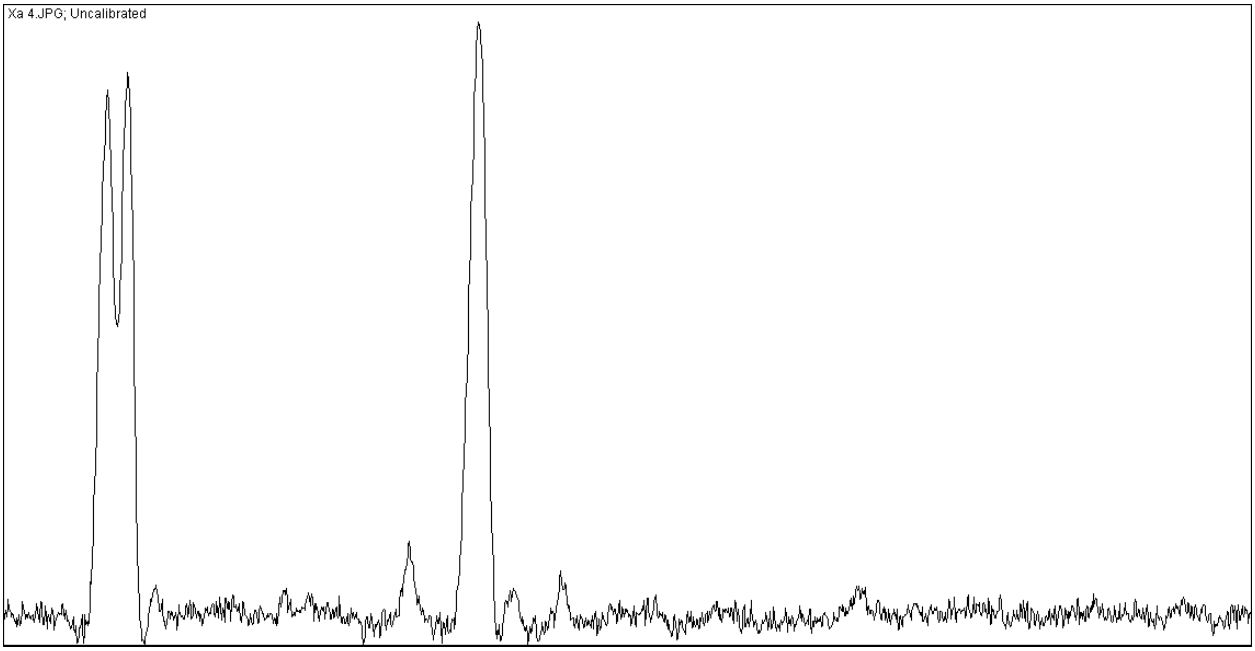
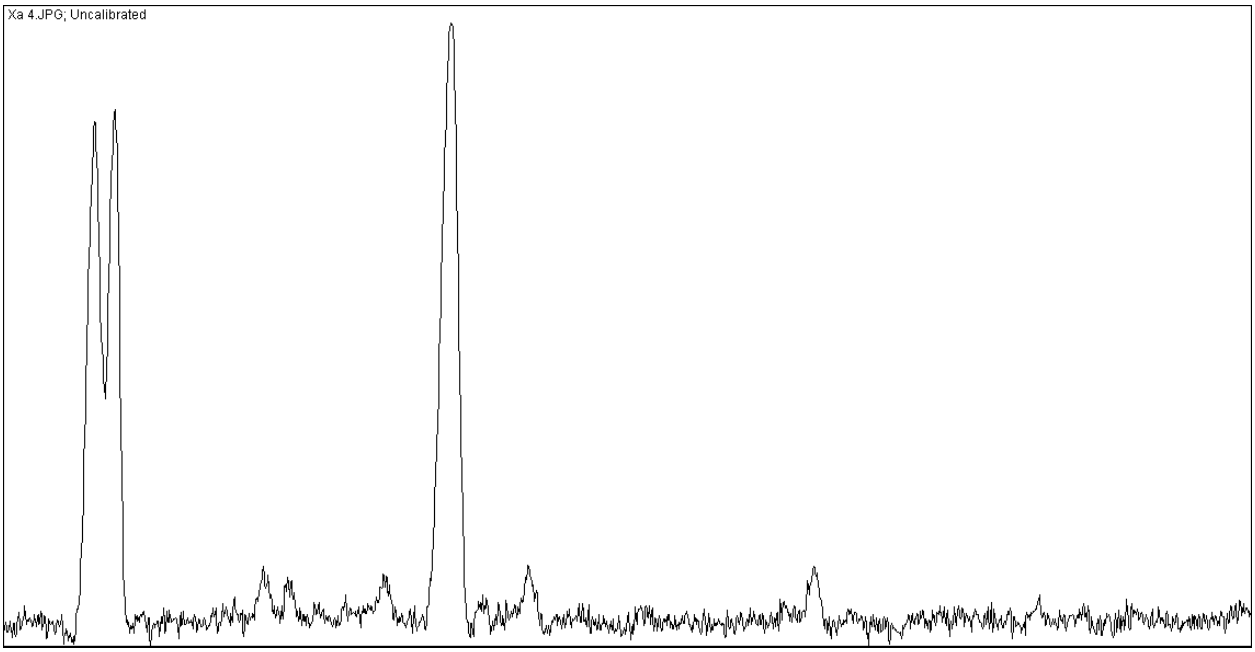
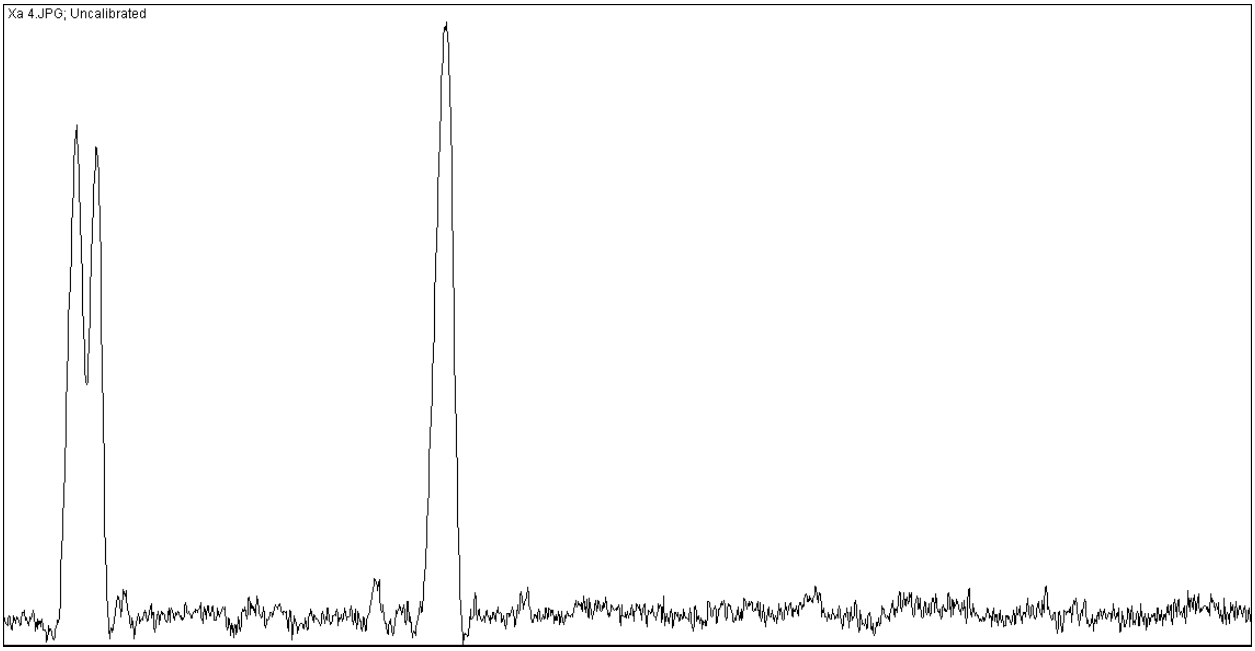


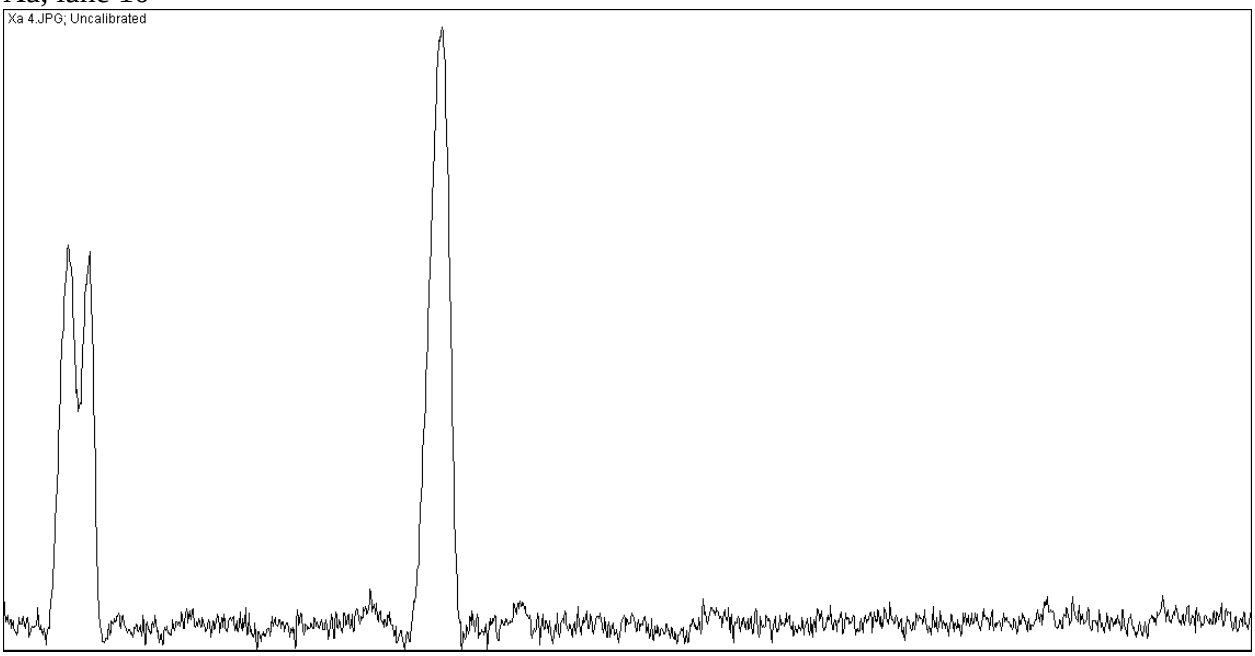
Xa, Lane
8



Xa, Lane
9

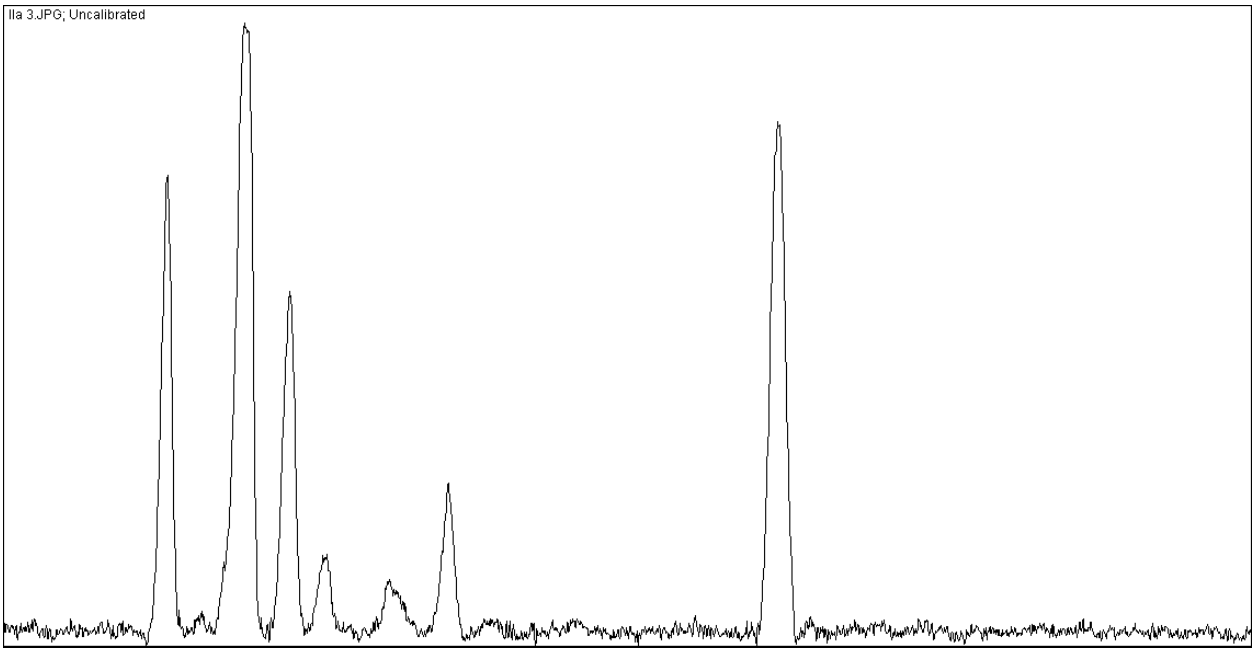


Xa, lane 10

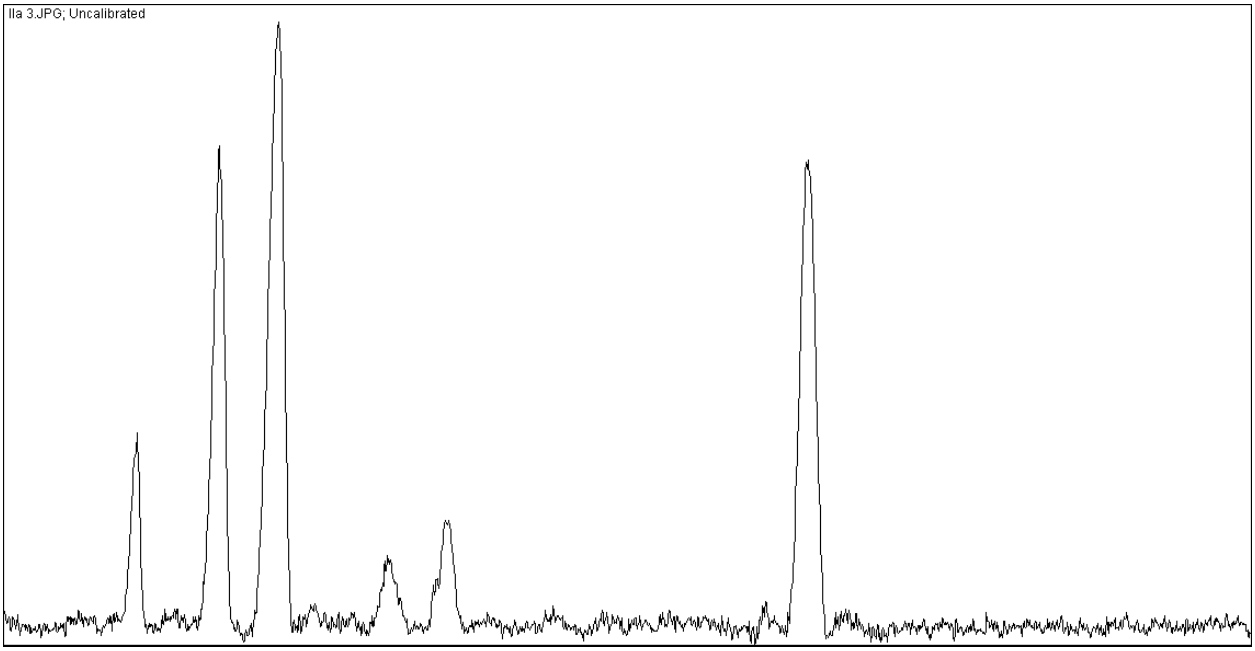


IIa examples

IIa, Lane
4



IIa, Lane
5



IIa, Lane
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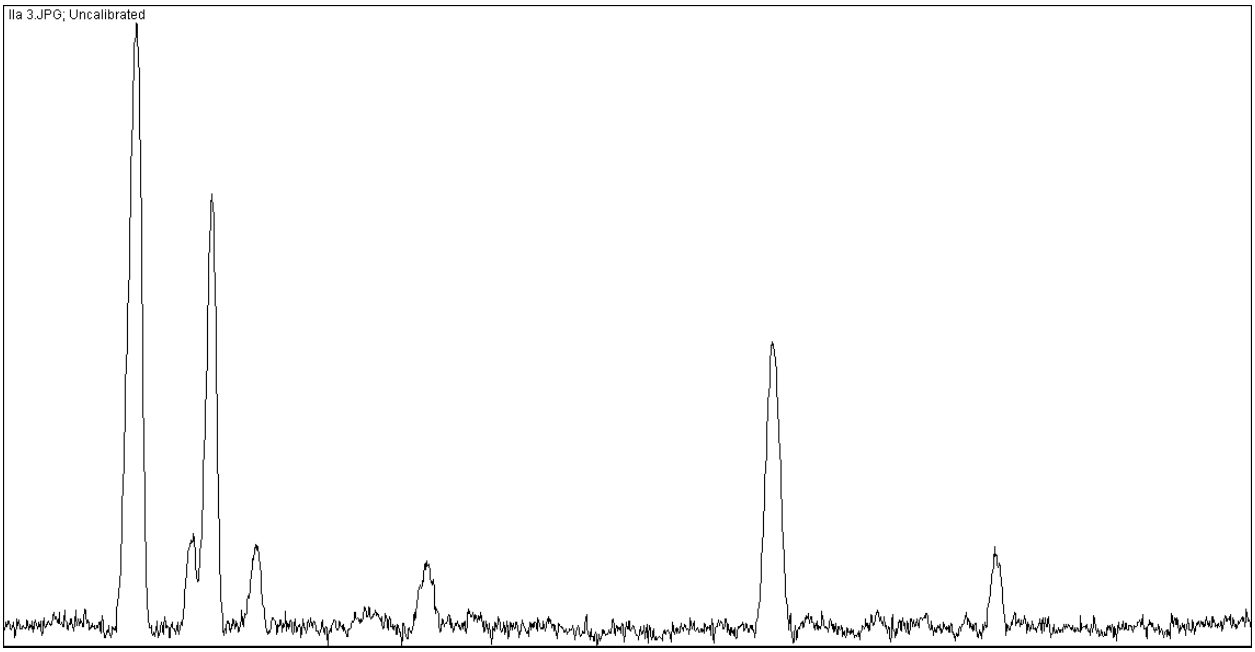

IIa, Lane
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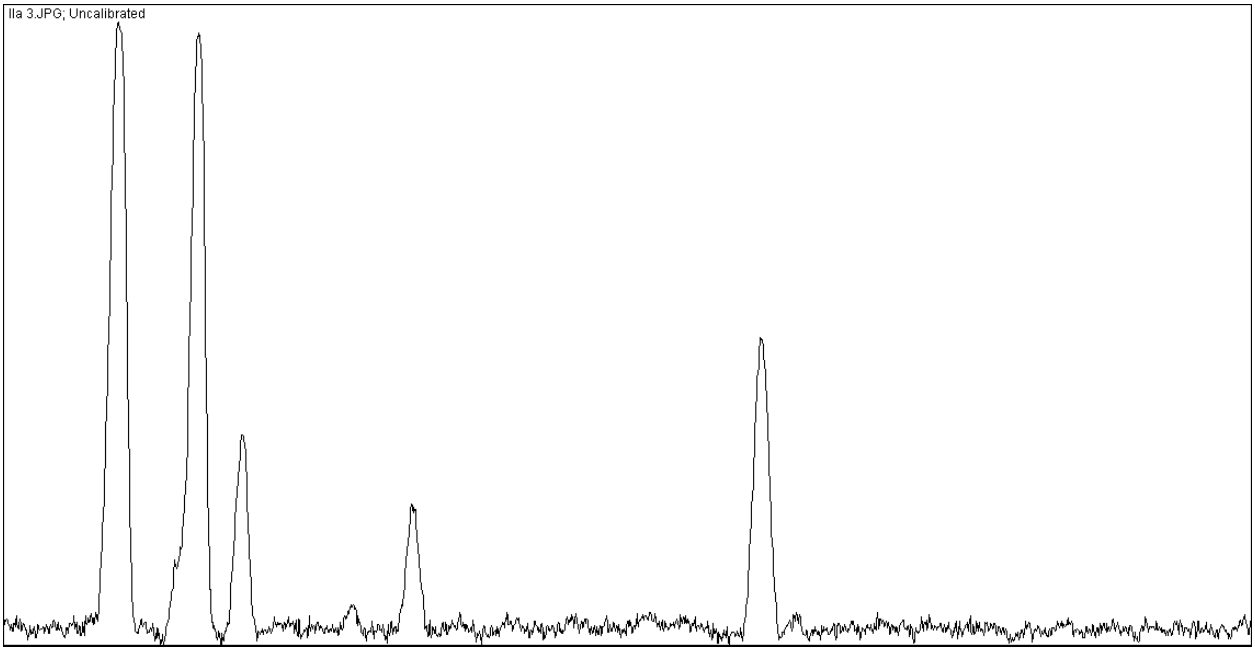

IIa, Lane
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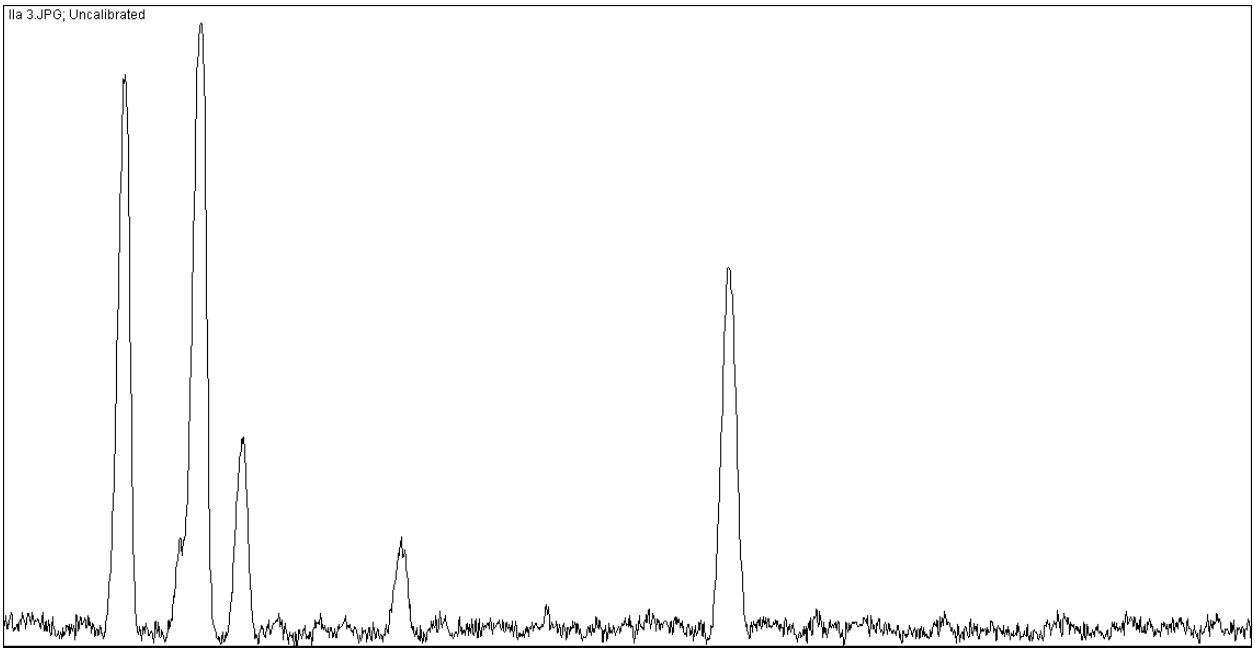

IIa, Lane
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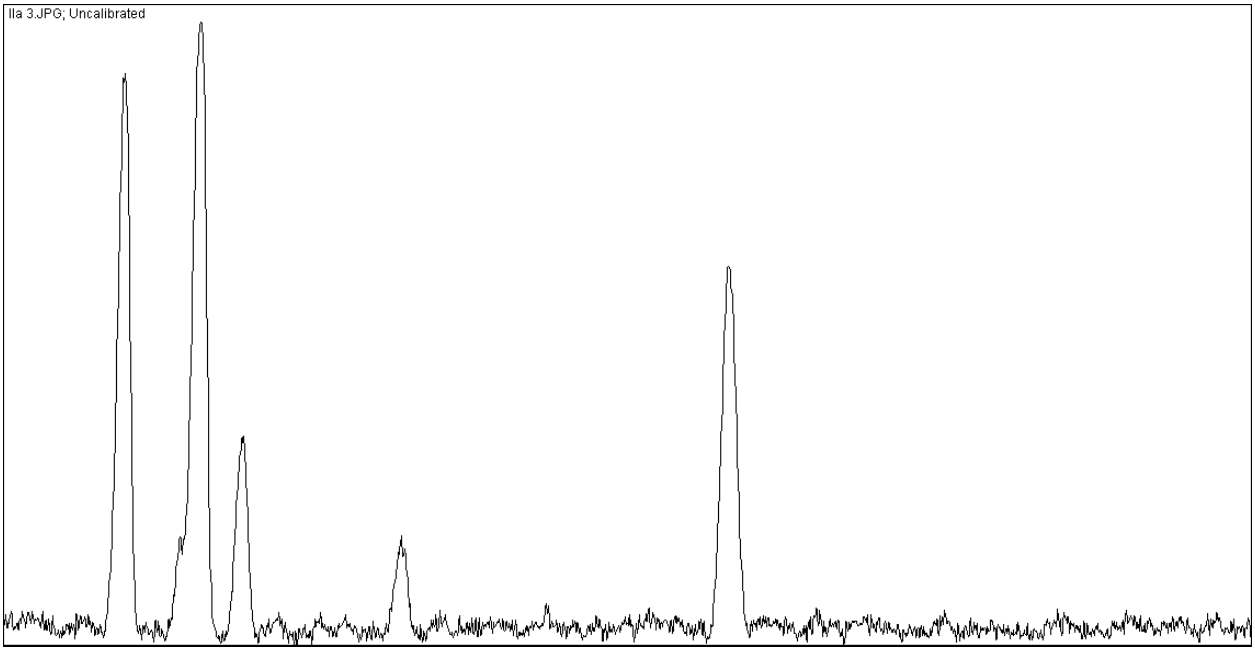
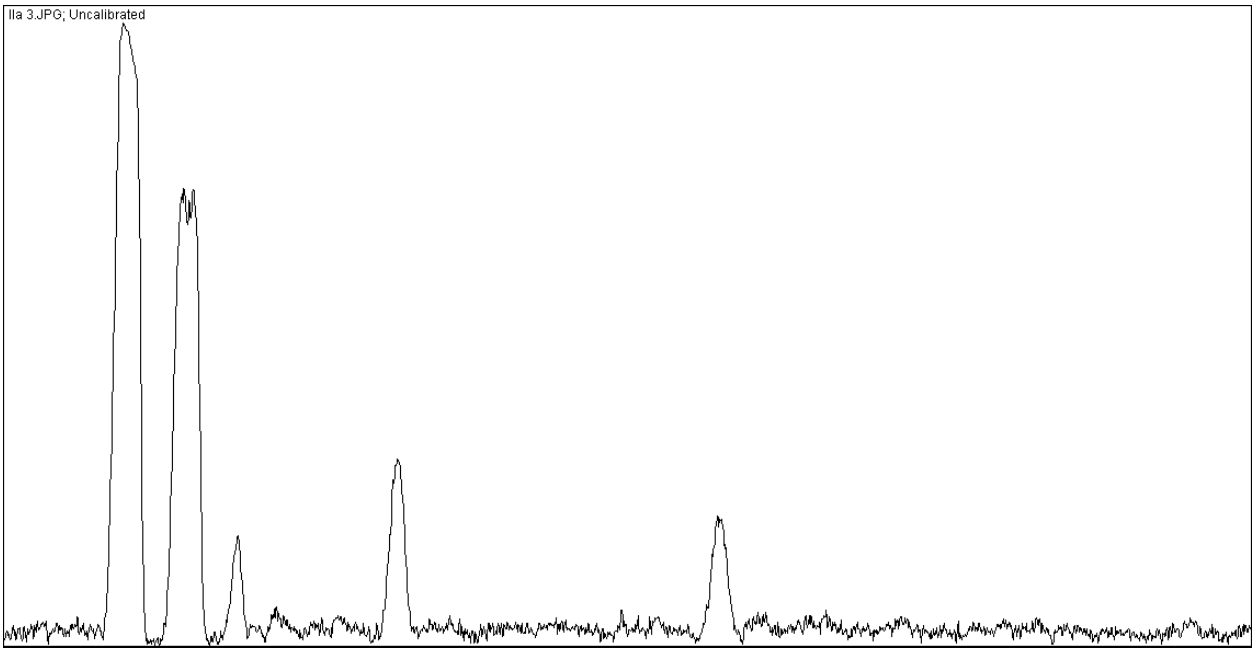
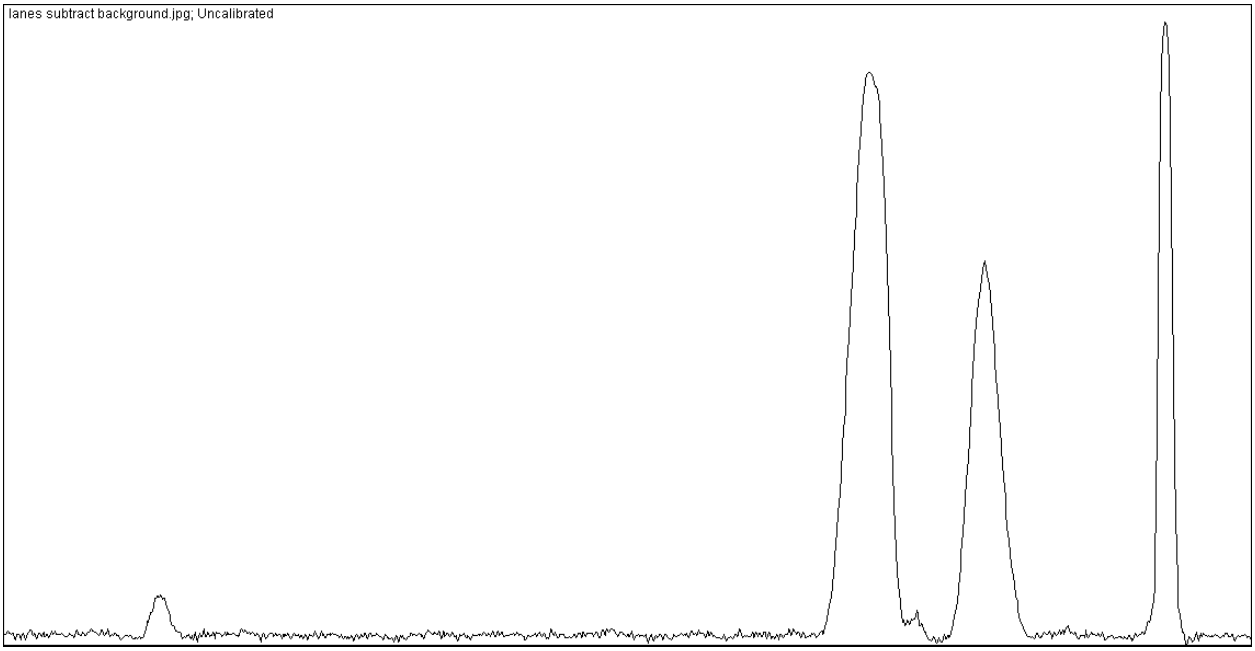


IIa, Lane
10

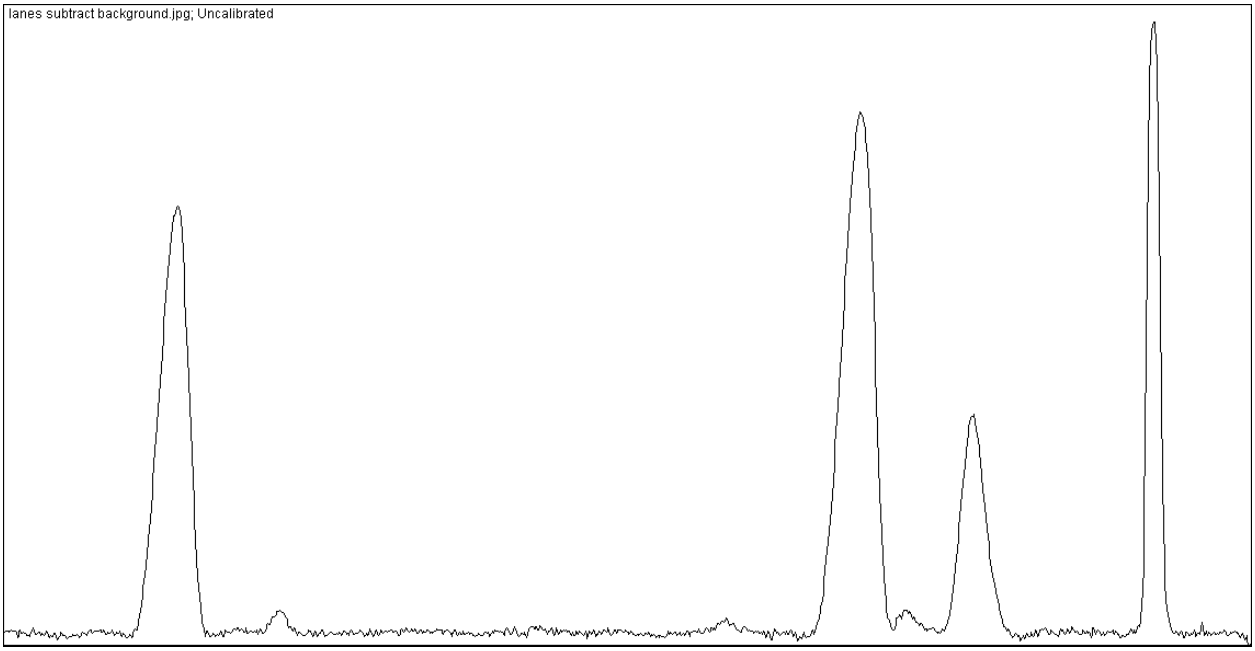


IXa examples

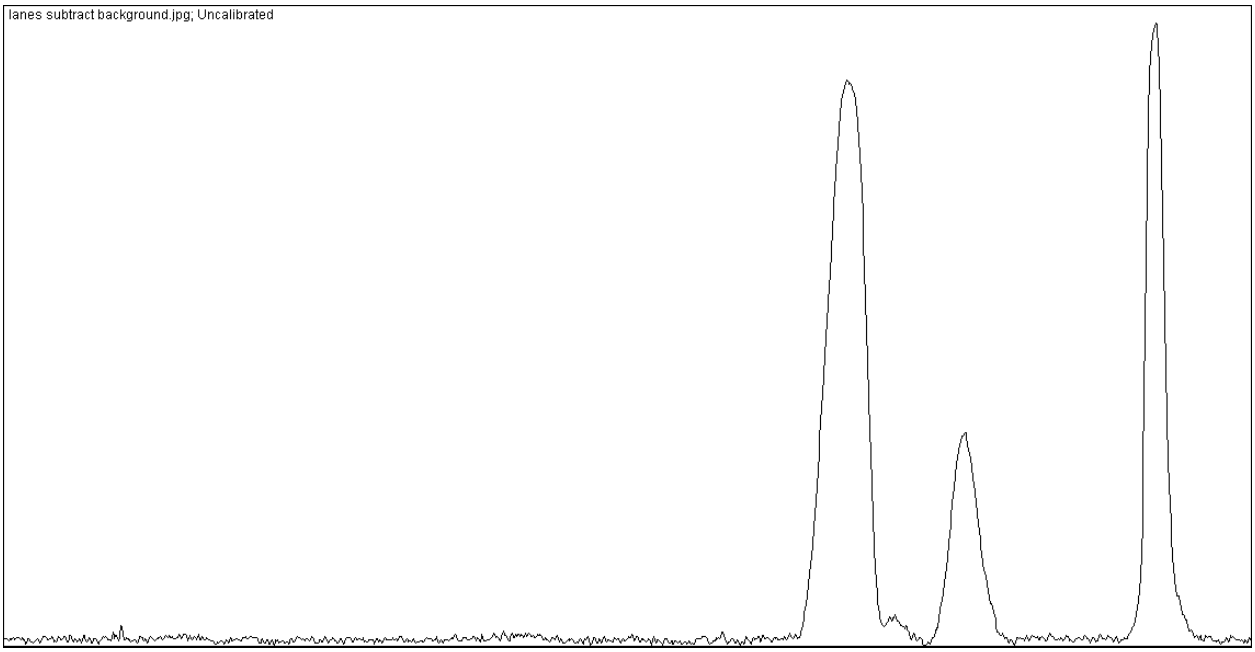
IXa, Lane
4



IXa, Lane
5



IXa, Lane
6



IXa, Lane
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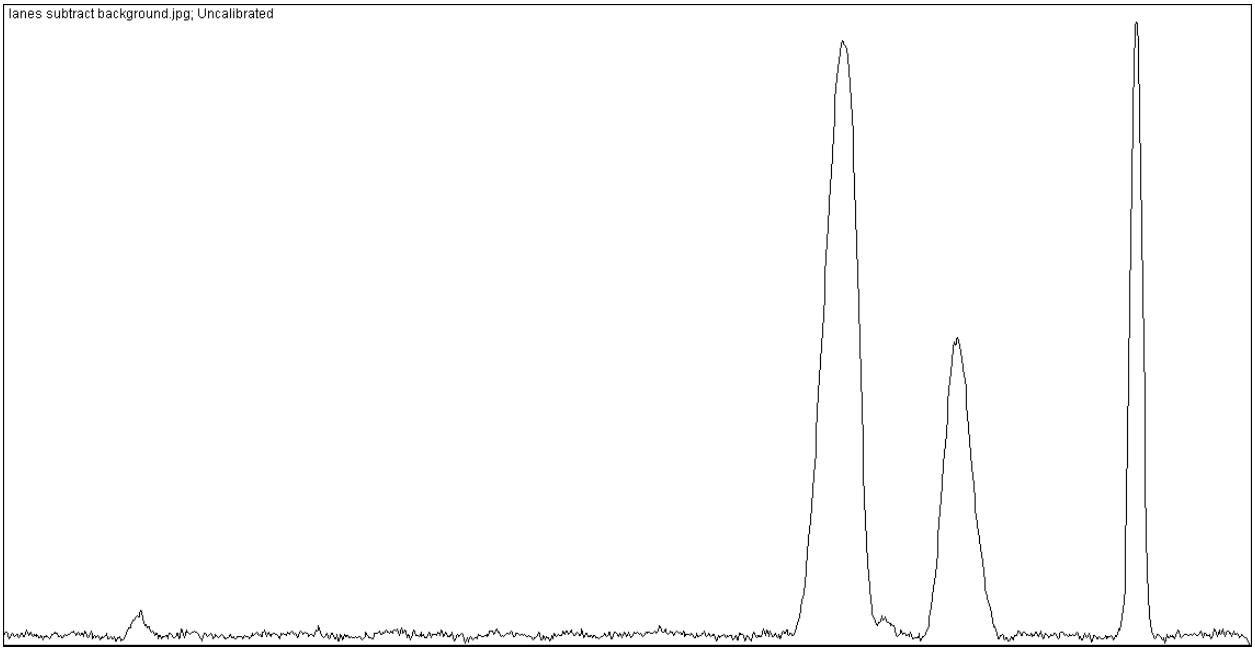

IXa, Lane
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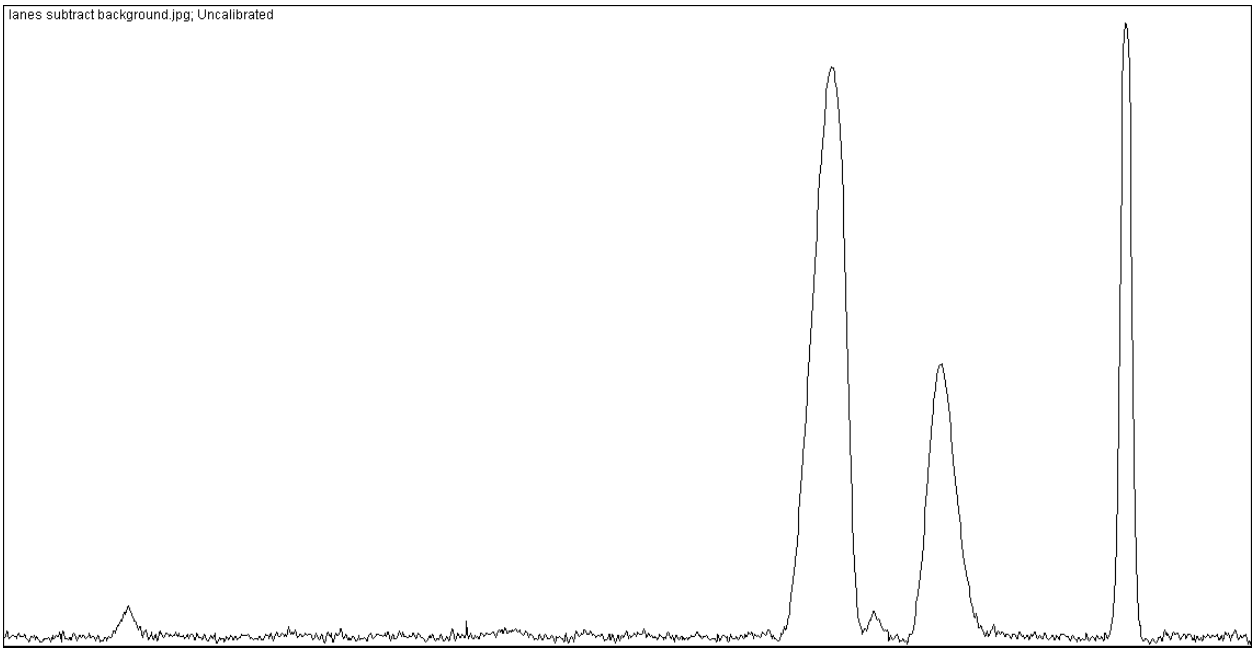

IXa, Lane
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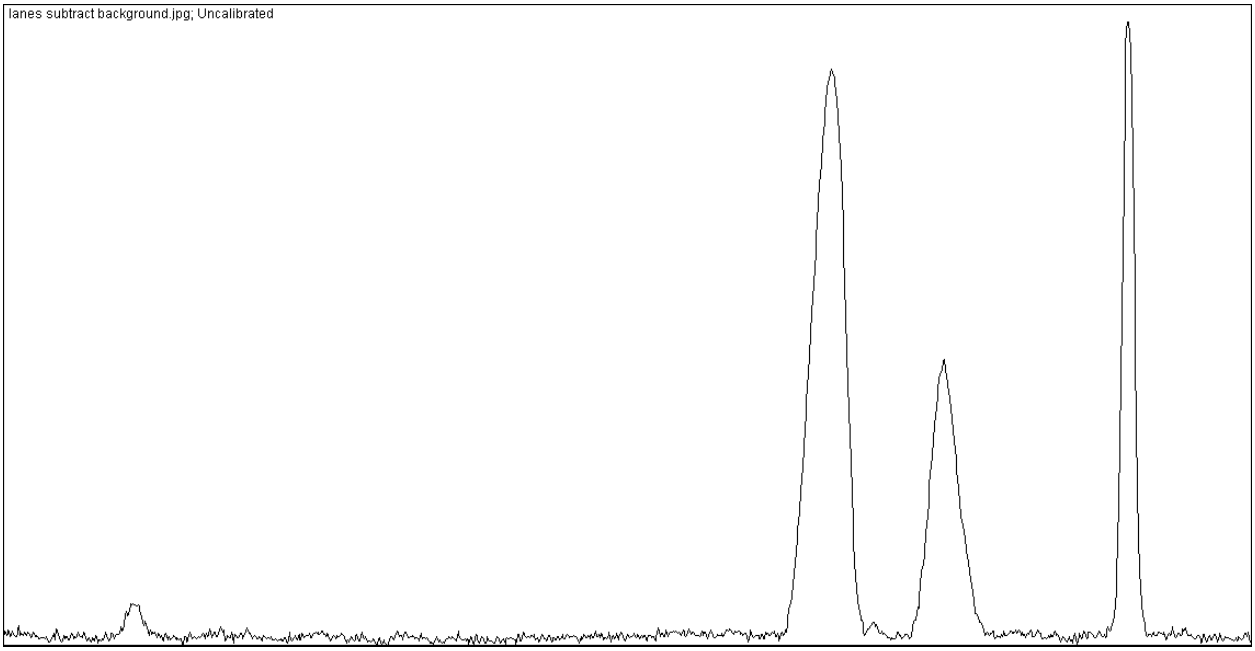

IXa, Lane
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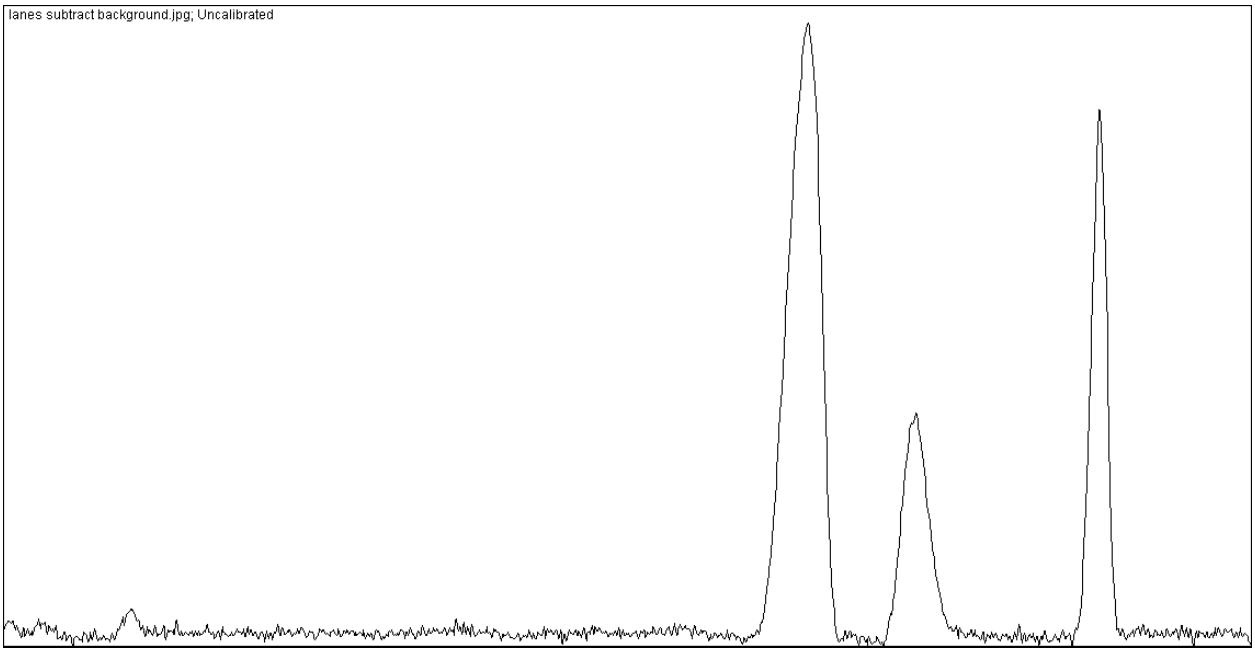

References

"Heparin." *TheFreeDictionary.com*. N.p., n.d. Web. 04Apr. 2013.

"Protamine Sulfate." *TheFreeDictionary.com*. N.p., n.d. Web. 004 Apr. 2013.

Rand, Margaret L, PhD, Elizabeth J Jarfeinst, PhD, & Rhobert K. McMurray, Md, PhD., "Plasma Proteins, Immunoglobulin, & Blood Coagulation" *Department of Biochemistry, University of Toranto.*

"Spermine, Spermidine, Putrescine." *BIOSYNTH*. N.p., 3 Apr. 2006. Web. 04 Apr. 2013.